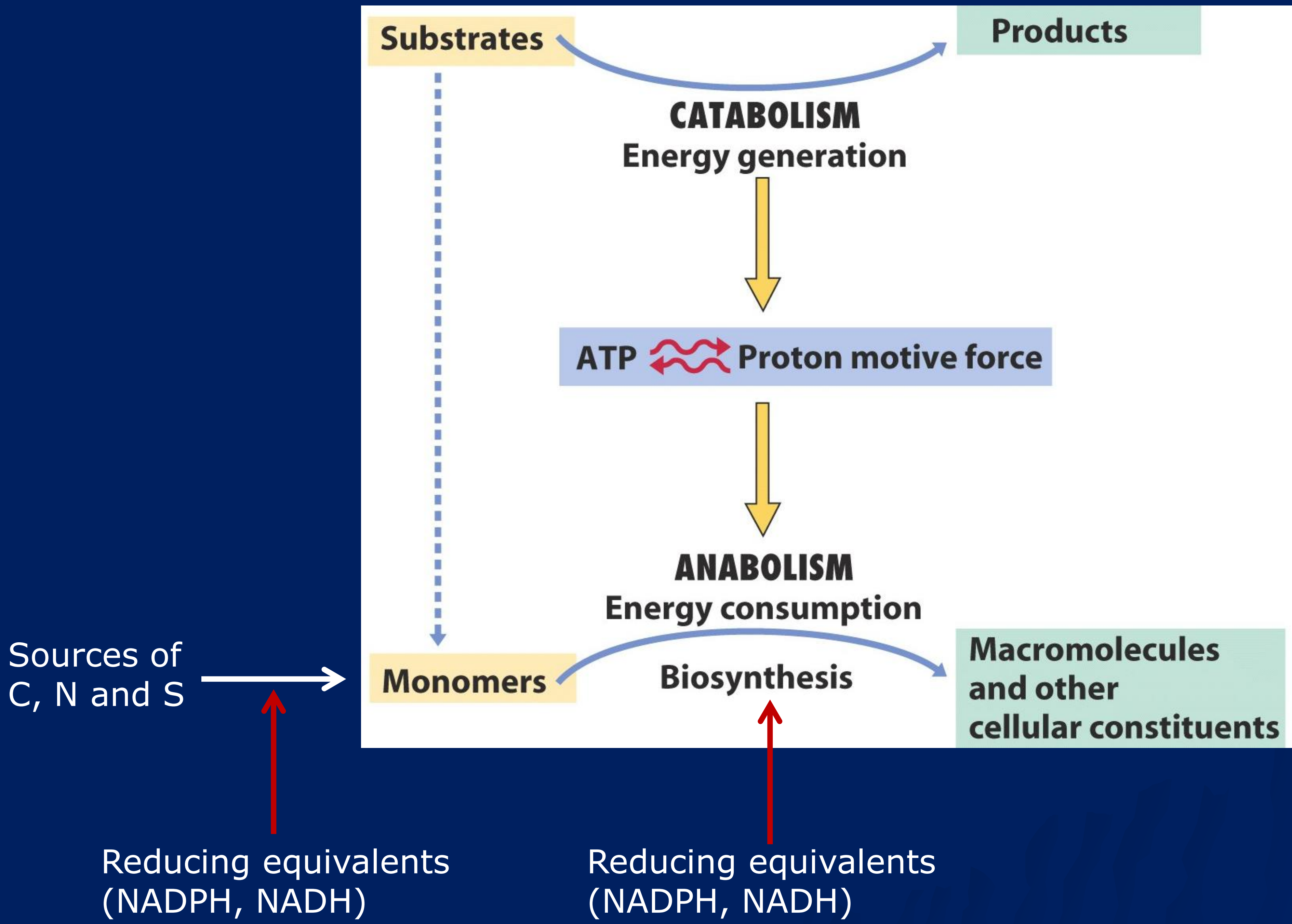


Substrates

Products

CATABOLISM

Energy generation

ATP ⇄ Proton motive force

ANABOLISM

Energy consumption

Monomers

Biosynthesis

Macromolecules and other cellular constituents

Sources of C, N and S

Reducing equivalents (NADPH, NADH)

Reducing equivalents (NADPH, NADH)

Ассимиляционные процессы у прокариот

Ассимиляция углерода

Метано- и метилотрофия как вариант углеродной хемолитотрофии с **восстановлением одноуглеродных соединений до формальдегида**

метилотрофы – общий класс гетеротрофных аэробов, которые могут утилизировать разные одноуглеродные молекулы – представители Pseudomonas, Bacillus, Vibrio.

Метанотрофы (Methylomonas, Methylococcus) имеют метан-монооксигеназу – широкая субстратная специфичность (метан, бромоэтан, этан, пропан, хлорометан, хрихлорэтилен и др.) может катализировать как окисление, так и восстановление.

$CH_4+O_2+\text{НАДН}\rightarrow CH_3\mathbf{OH}+H_2O+\text{НАД}+$ (монооксигеназа)

$CH_4+O_2+\text{цит.с восст}\rightarrow CH_3\mathbf{OH}+H_2O+\text{цит.с окисл}$

- Спирты, которые образуются могут использоваться для образования формальдегида $CH_3\mathbf{OH}+X \rightarrow H\mathbf{CHO}+XH_2$ (метанолДГ)
- Далее возможны 2 реакции (последовательное окисление формальдегида до CO_2)

  $HCHO+X+H_2O \rightarrow HCOOH+XH_2$ (формальд-дДГ)

  $HCOOH+$НАД$\rightarrow CO_2+$НАДН (формиатДГ)

Формальдегид может использоваться

У метанотрофов для синтеза глицеральдегида

- рибулозо-монофосфатный цикл : синтез глицеральдегида из 3х молекул формальдегида с затратой АТФ (Methylomonas, Methylococcus)

у метилотрофов **формальдегид** используется для синтеза **серина**

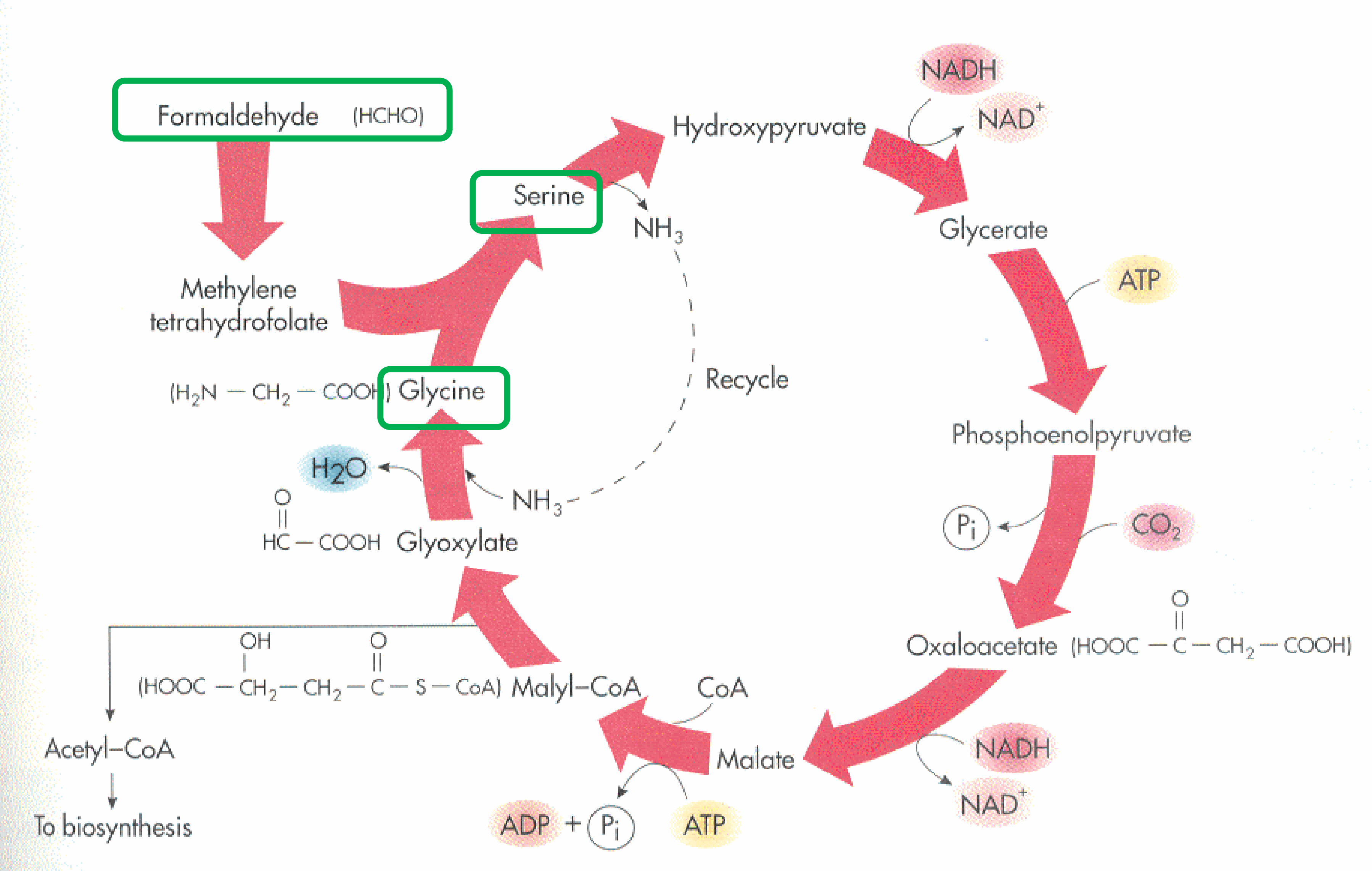
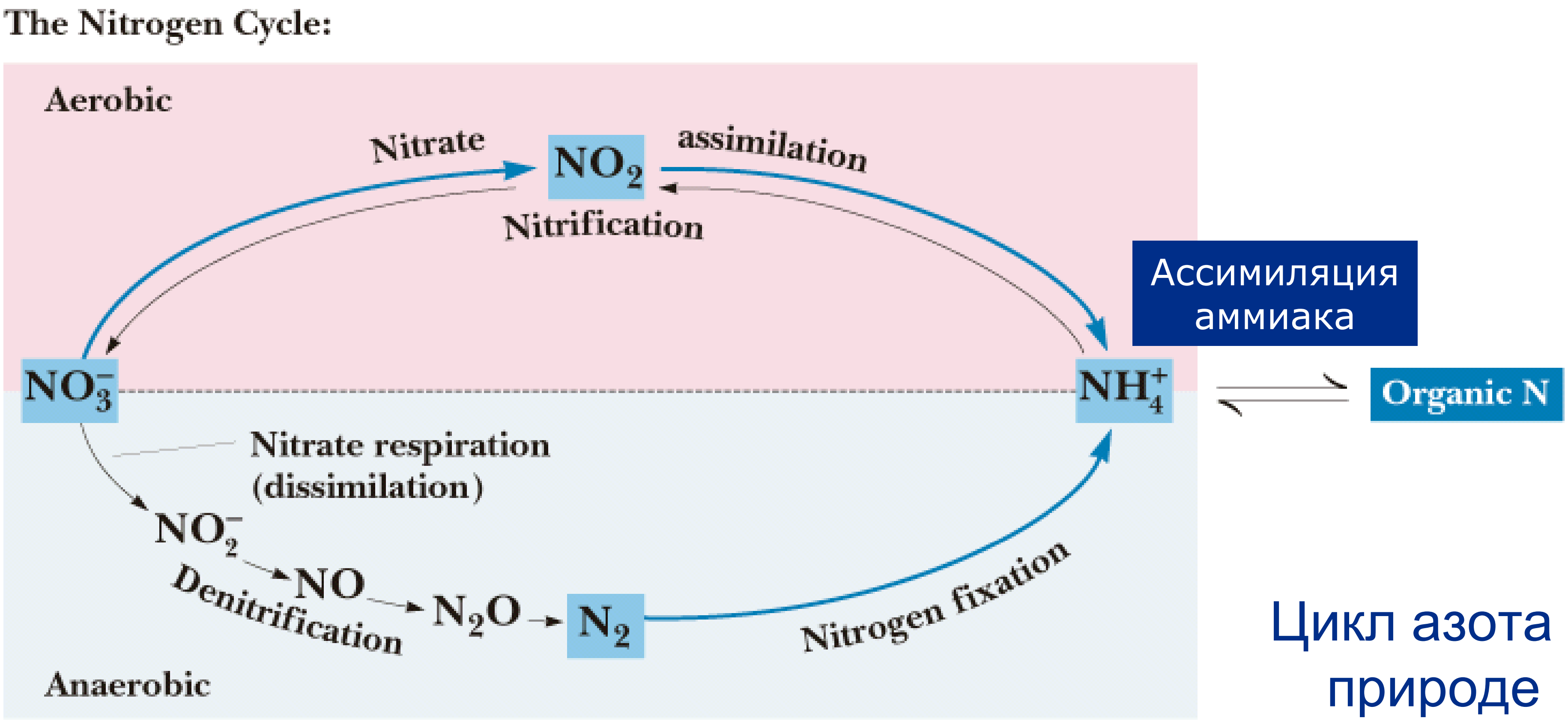


Fig. 5-10 Serine Pathway of Methylotrophic Bacteria. The serine pathway for the assimilation of C-1 units into cell material by Type II methylotrophic bacteria.

Ассимиляционные процессы у прокариот

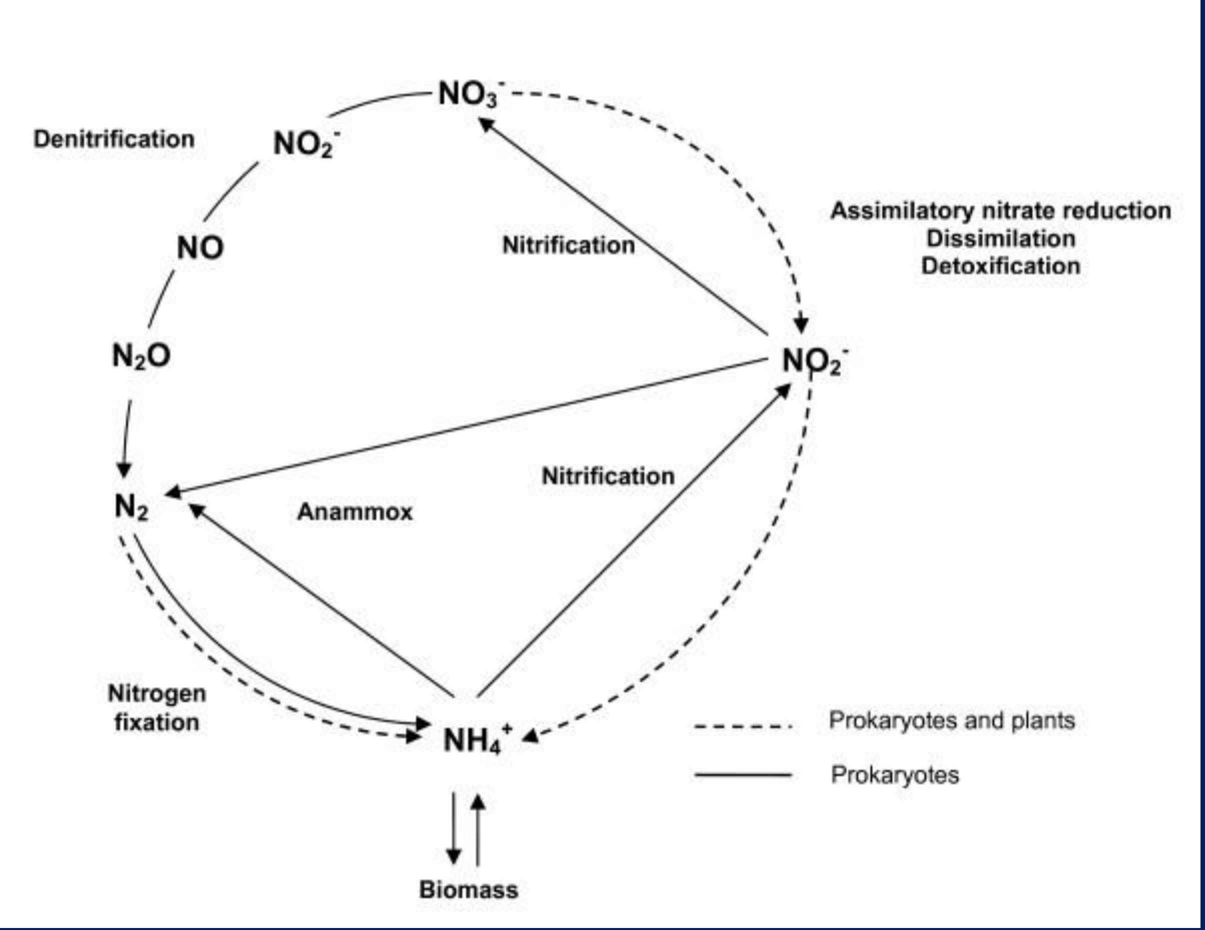
Ассимиляция азота:
включение азота аммиака в состав амино- и амидо-групп аминокислот



Ассимиляция аммиака

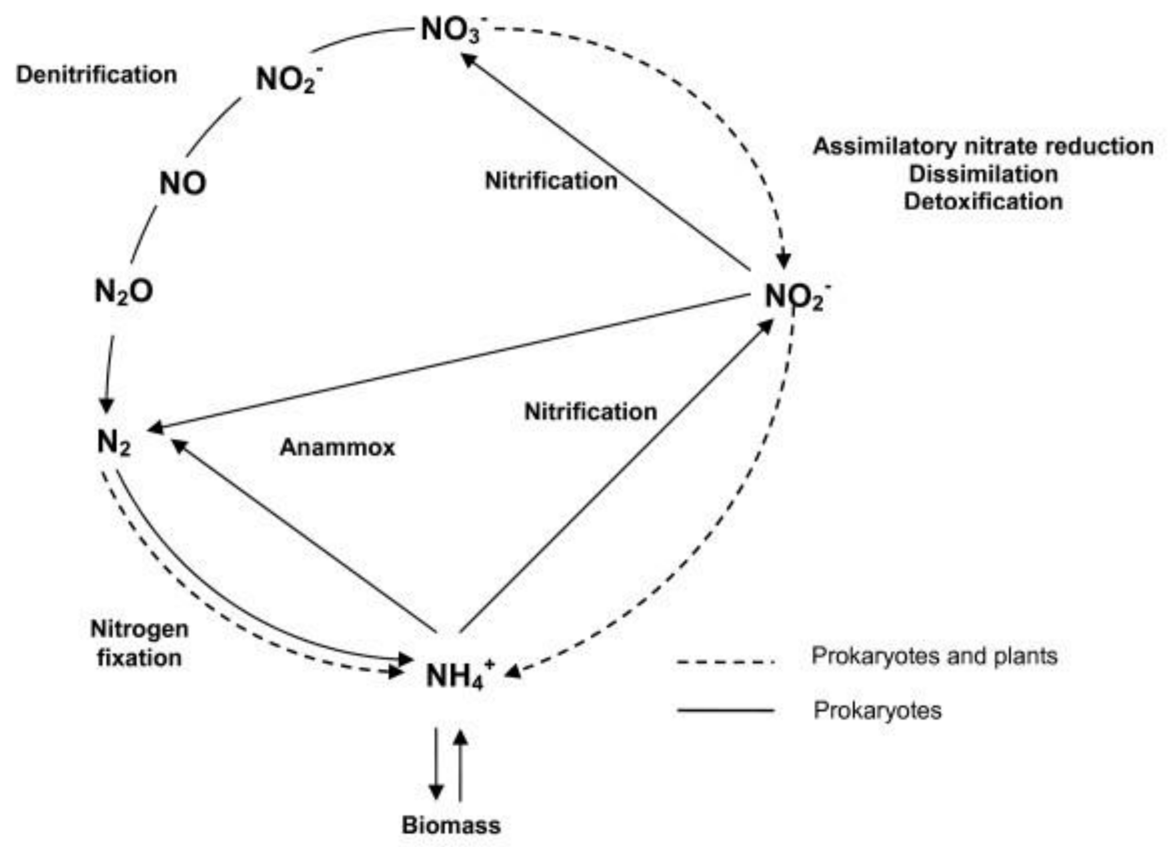
Цикл азота в природе

N-cycle scheme



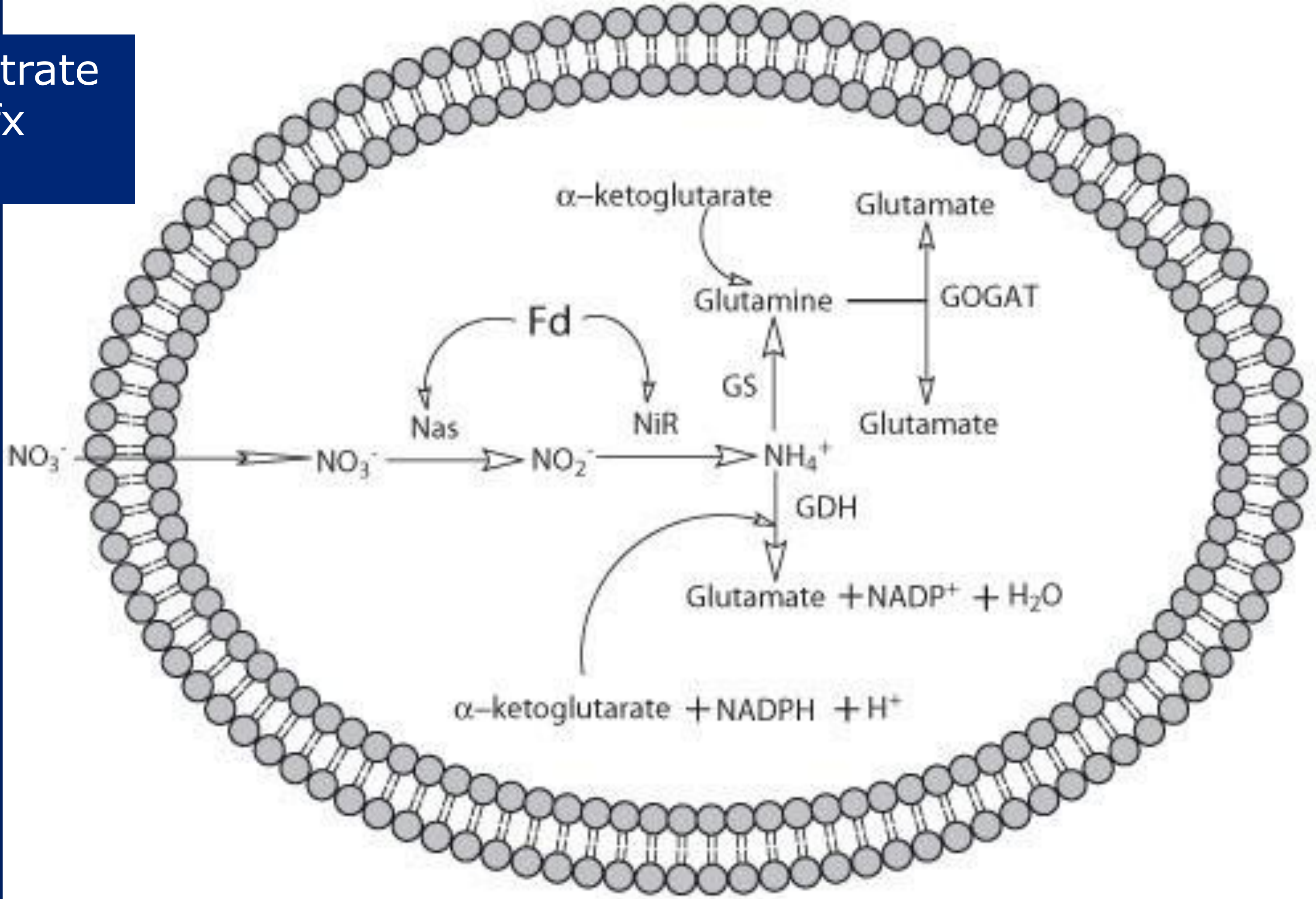
- NO_3^- is used as nitrogen source for growth under aerobic conditions using an *assimilatory NO_3^- reductase*, while it acts as an electron acceptor to eliminate excess of reductant power through *dissimilatory NO_3^- reduction*.
- *Dissimilatory NO_3^- reduction*, *NO_3^- respiration* or *denitrification* are often used equivalently in the literature. However, *dissimilatory* pathway makes reference to non-assimilatory reactions that are not directly coupled to generation of proton-motive force.

N-cycle scheme

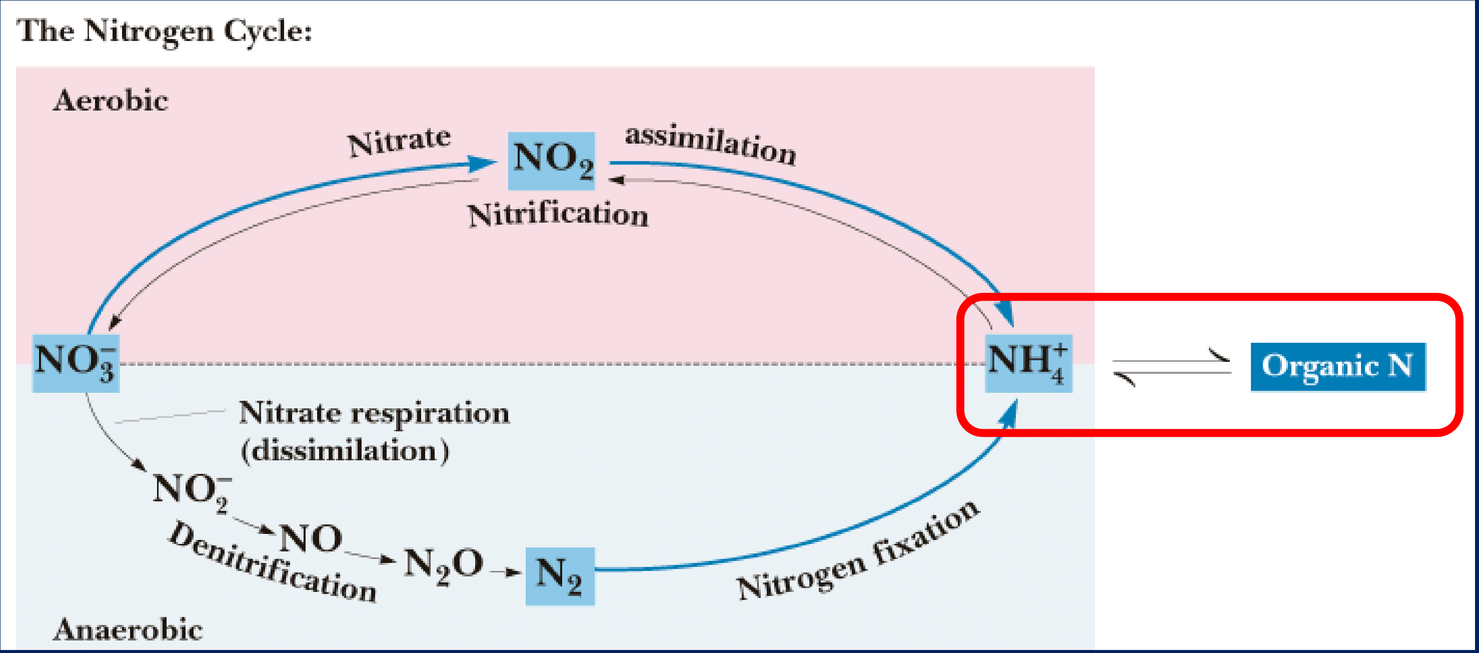


- In some Enterobacteriaceae, **NO_2^- is reduced to NH_4^+ which is then excreted**; this process is known as *NO_3^-/NO_2^-* ***ammonification***.
- Specialised organisms are able to oxidize either NH_4^+ or NO_2^- by using a pathway called *nitrification*, while other organisms such as some planctomycetes oxidize NH_4^+ and utilize NO_2^- as respiratory electron acceptor in a pathway named *anammox*. Finally, *(di)nitrogen fixation* allows to reduce N_2 to NH_4^+ to provide N-requirements.

Assimilatory nitrate reduction in Hfx mediterranei



Nas: assimilatory nitrate reductase;
NiR: assimilatory nitrite reductase;
Fd: Ferredoxin;
GDH: glutamate dehydrogenase;
GS: glutamine synthetase;
GOGAT: glutamate synthase.



The Fate of Ammonium

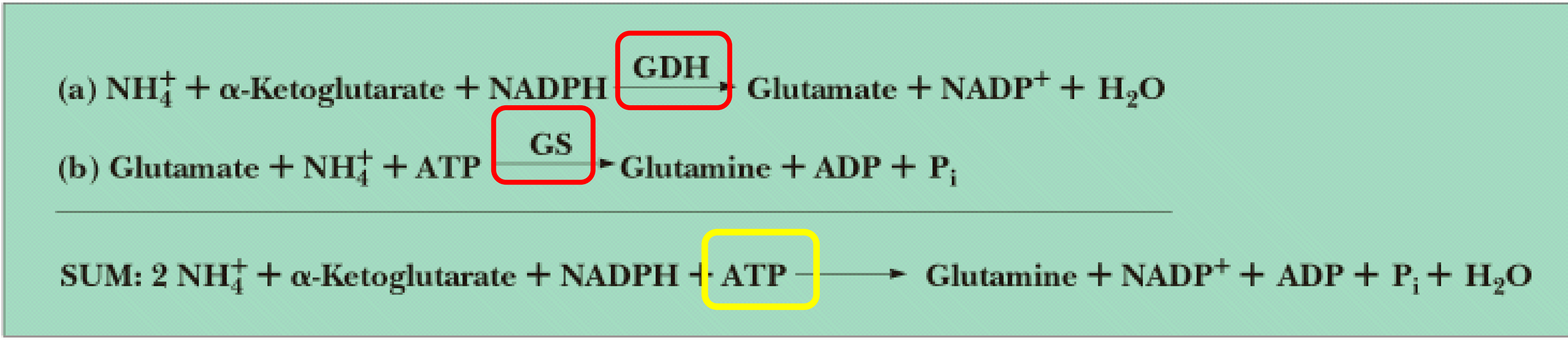
- Carbamoyl-phosphate synthetase I
  - two ATP required - one to activate CO2, one to phosphorylate carbamate
- Glutamate dehydrogenase
  - reductive amination of alpha-ketoglutarate to form glutamate
- Glutamine synthetase
  - ATP-dependent amidation of gamma-carboxyl of glutamate to glutamine

При высоких концентрациях аммиака

с участием глутаматДГ (GDH) и

далее глутамин-синтетазы (GS).

Нужны 1АТФ и 1NADPH



(a) $NH_4^+ + \alpha\text{-Ketoglutarate} + NADPH \xrightarrow{\boxed{GDH}} \text{Glutamate} + NADP^+ + H_2O$

(b) $\text{Glutamate} + NH_4^+ + ATP \xrightarrow{\boxed{GS}} \text{Glutamine} + ADP + P_i$

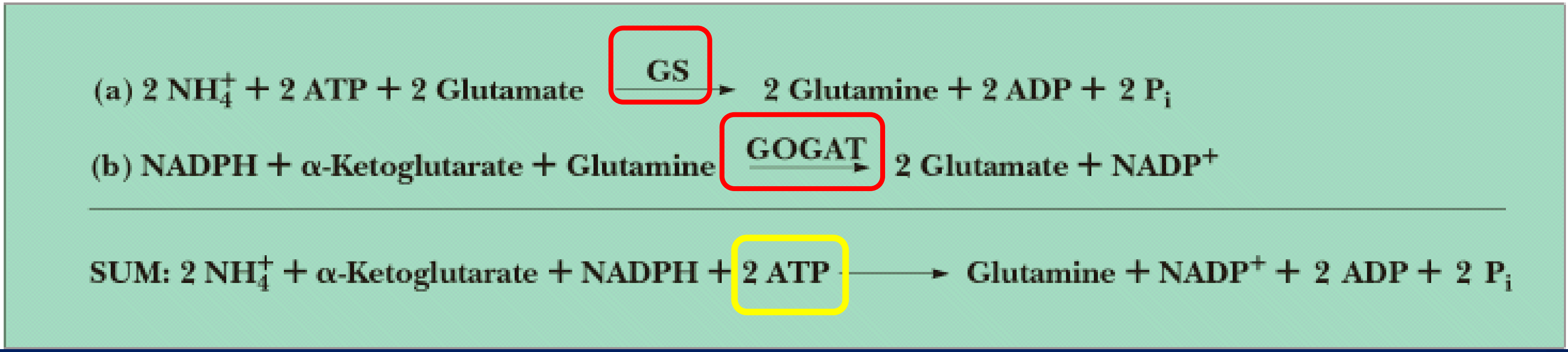
SUM: $2\ NH_4^+ + \alpha\text{-Ketoglutarate} + NADPH + \boxed{ATP} \longrightarrow \text{Glutamine} + NADP^+ + ADP + P_i + H_2O$

При низких концентрациях аммиака

с участием глутамин-синтетазы (GS) и

далее глутамин-оксоглутарат аминотрансферазы (GOGAT).

Нужны **2** АТФ и 1NADPH



$$\text{(a) } 2\,\mathrm{NH_4^+} + 2\,\mathrm{ATP} + 2\,\text{Glutamate} \boxed{\xrightarrow{\mathrm{GS}}} 2\,\text{Glutamine} + 2\,\mathrm{ADP} + 2\,\mathrm{P_i}$$

$$\text{(b) } \mathrm{NADPH} + \alpha\text{-Ketoglutarate} + \text{Glutamine} \boxed{\xrightarrow{\mathrm{GOGAT}}} 2\,\text{Glutamate} + \mathrm{NADP^+}$$

$$\text{SUM: } 2\,\mathrm{NH_4^+} + \alpha\text{-Ketoglutarate} + \mathrm{NADPH} + \boxed{2\,\mathrm{ATP}} \longrightarrow \text{Glutamine} + \mathrm{NADP^+} + 2\,\mathrm{ADP} + 2\,\mathrm{P_i}$$

Ассимиляционные процессы у прокариот

- **Ассимиляция серы**
  восстановление сульфата до сульфида и далее включение серы в цистеин через образование аденозинфосфосульфата (АФС) и тиоредоксина

ATP+SO_4^{-2} —*(АТР-сульфурилаза)*→ Аденозин 5'-фосфосульфат+PPi.

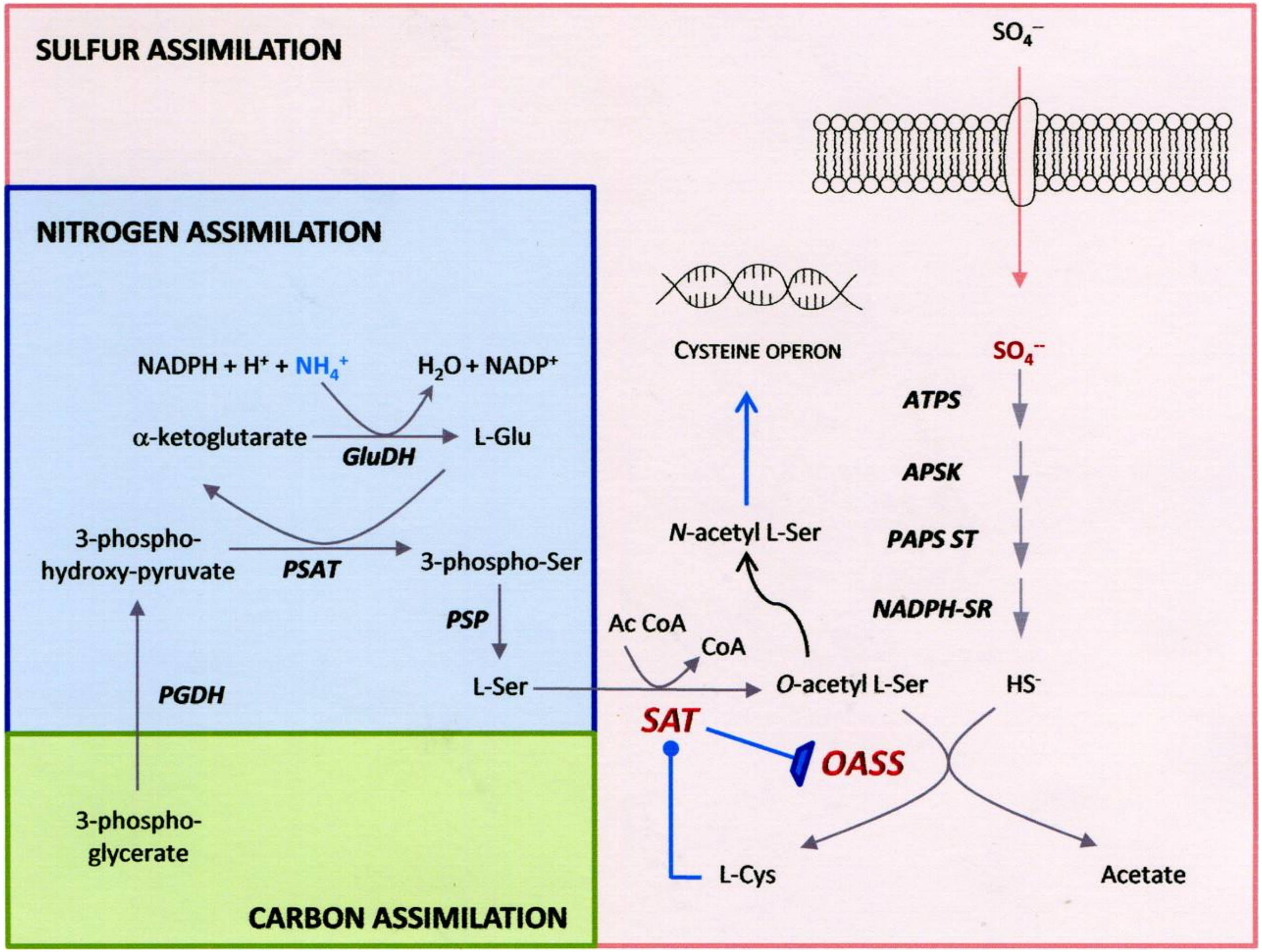
ATP+АФС —*(АФС-фосфокиназа)*→ Аденозин -3 -Ф 5'-фосфосульфат (ФАФС) +АДР

2R—SH+ФАФС —*(ФАФС редуктаза)*——→ Сульфит+AMP-3′Ф+R—SS—R
(R-SH — тиоредоксин; восстановленная: форма регенерируется из окисленной)

Сульфит+3NADPH — *(сульфитредуктаза)*——→ H_2S+3NADP

О-ацетил-L-серин+H_2S –*(О-ацетилсерин ацетатлиаза)*→ L-цистеин+Ацетат+H_2O

Reductive sulfate assimilation pathway in bacteria and its relations with the nitrogen and carbon assimilation pathways.



Salsi E et al. J. Biol. Chem. 2010;285:12813-12822

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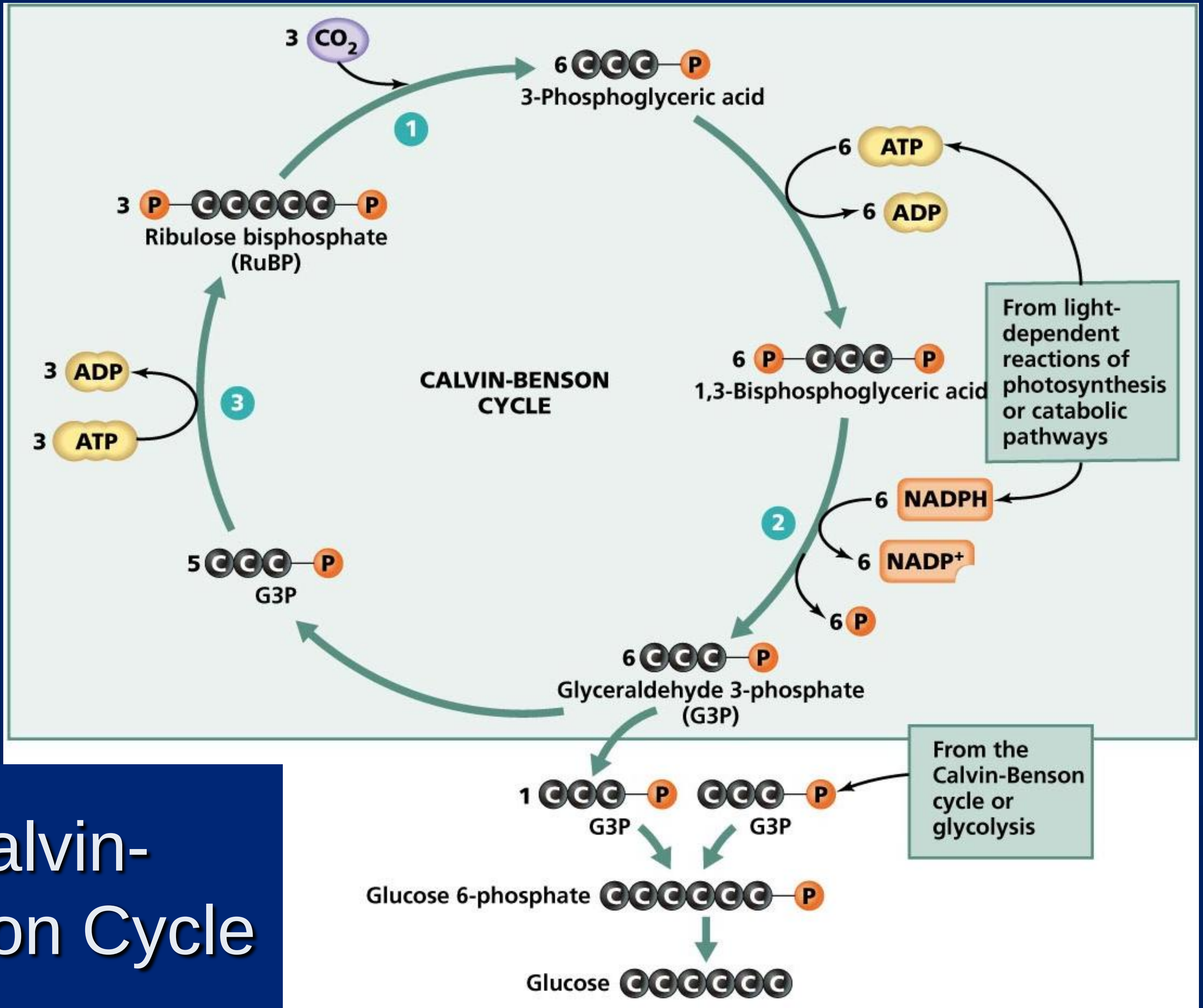
Фиксация углерода

1) цикл Кальвина (рибулозо-бисфосфатный цикл с ключевым ферментом RUBISCO)



Calvin-Benson Cycle

- 2) восстановительный ЦТК

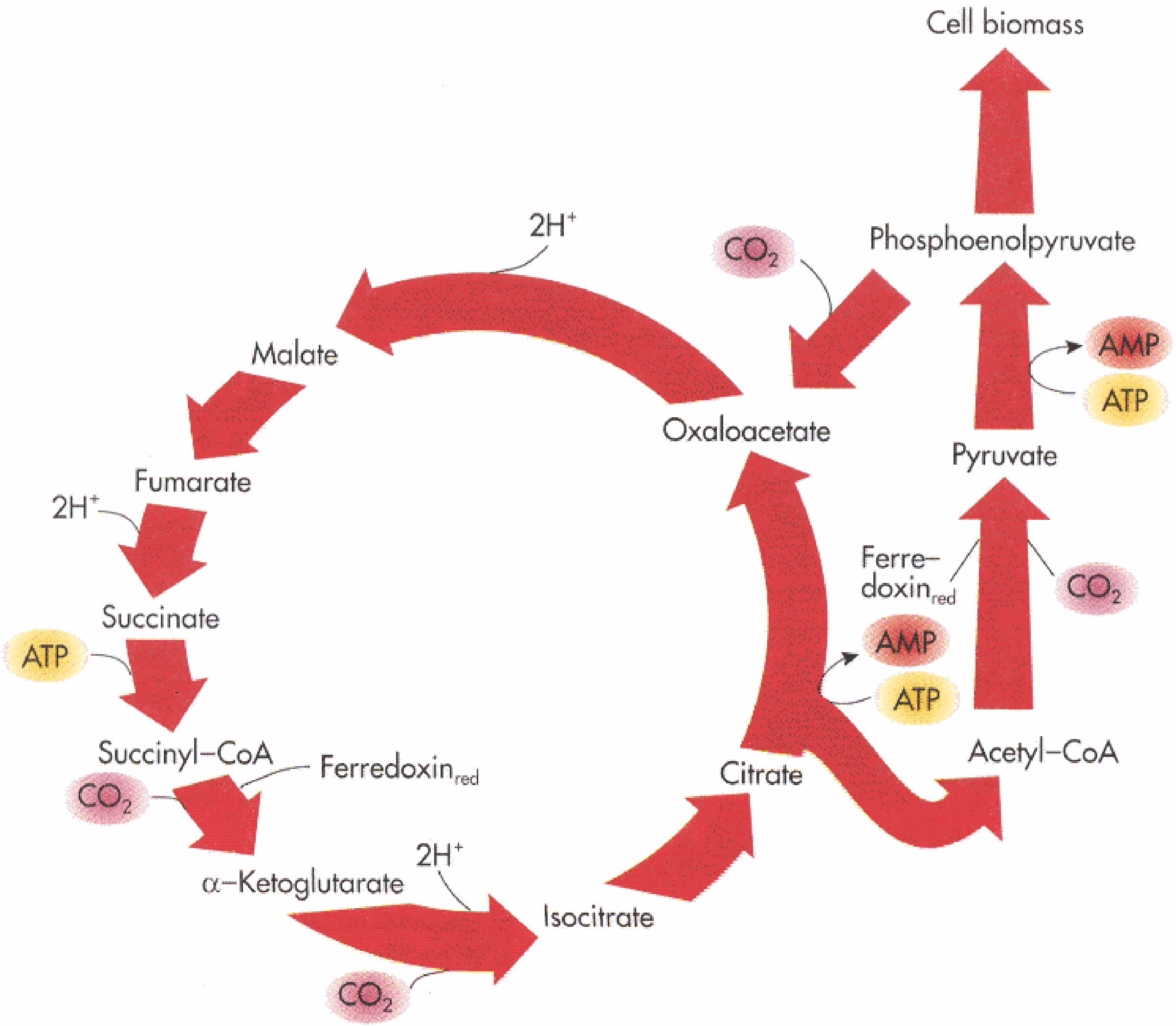


Fig. 5-6 Reductive Tricarboxylic Acid Cycle. The reductive tricarboxylic acid cycle of some photoautotrophs converts CO_2 molecules into an oxaloacetate molecule for incorporation in cell biomass.

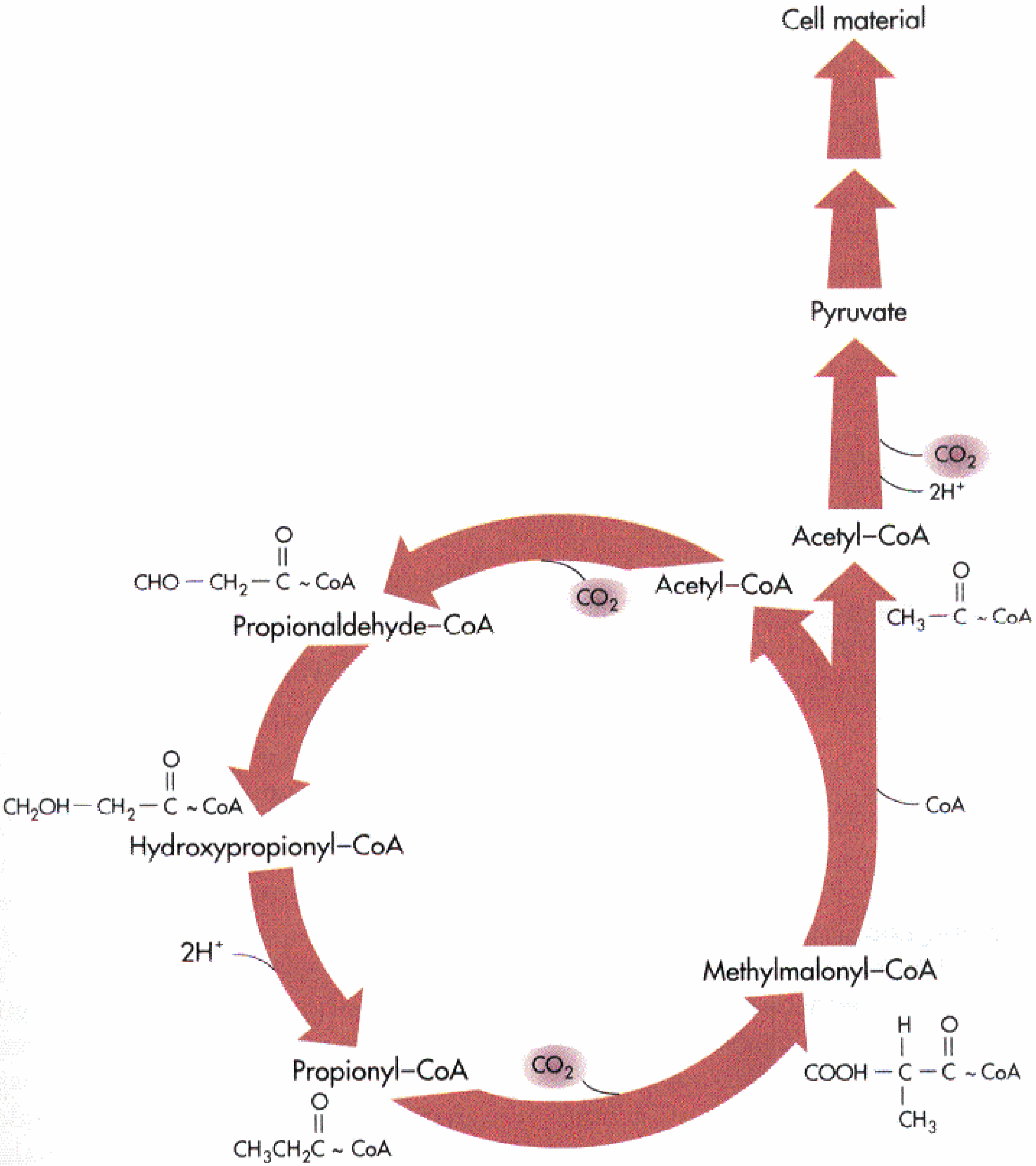

Cell material
Pyruvate
CO_2
$2H^+$
Acetyl–CoA
Acetyl–CoA
CO_2
$CH_3-\overset{O}{\overset{\|}{C}}\sim CoA$
$CHO-CH_2-\overset{O}{\overset{\|}{C}}\sim CoA$
Propionaldehyde–CoA
$CH_2OH-CH_2-\overset{O}{\overset{\|}{C}}\sim CoA$
Hydroxypropionyl–CoA
CoA
$2H^+$
Methylmalonyl–CoA
$COOH-\overset{H}{\overset{|}{\underset{\underset{CH_3}{|}}{C}}}-\overset{O}{\overset{\|}{C}}\sim CoA$
Propionyl–CoA
CO_2
$CH_3CH_2\overset{O}{\overset{\|}{C}}\sim CoA$

Fig. 5-7 Hydroxypropionate Pathway. The hydroxypropionate pathway of *Chloroflexus* converts CO_2 into acetyl-CoA for incorporation into cell biomass.

- 3) гидрокси-пропионатный путь фиксации С

4) Фиксация углерода в реакциях карбоксилирования пирувата

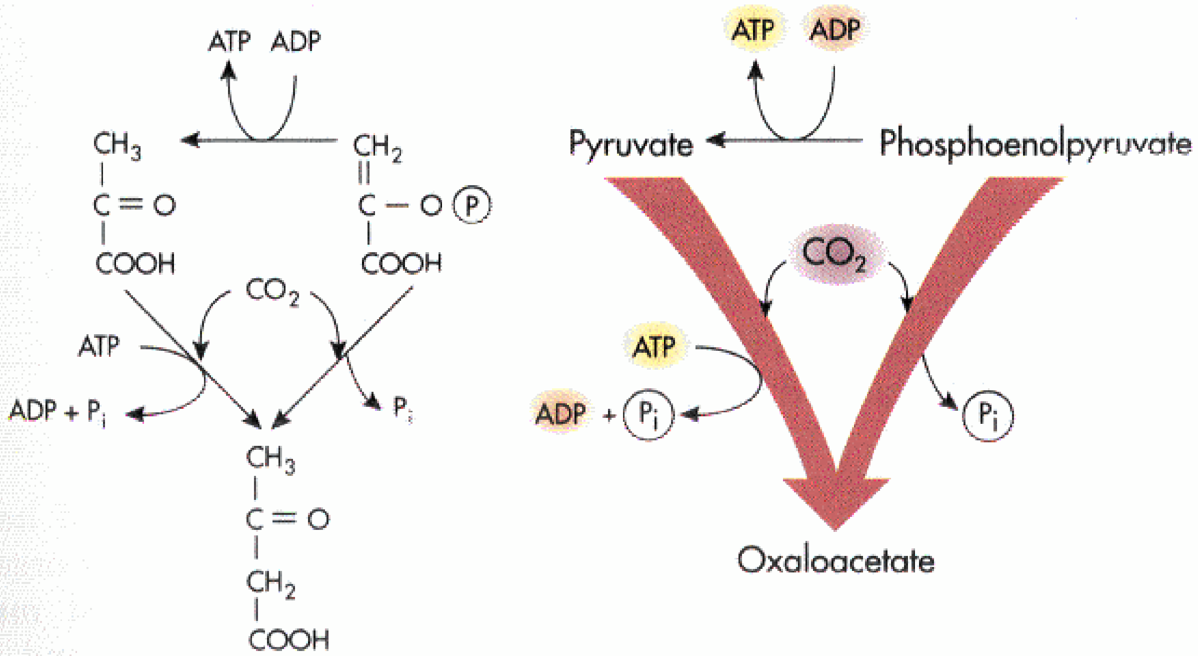
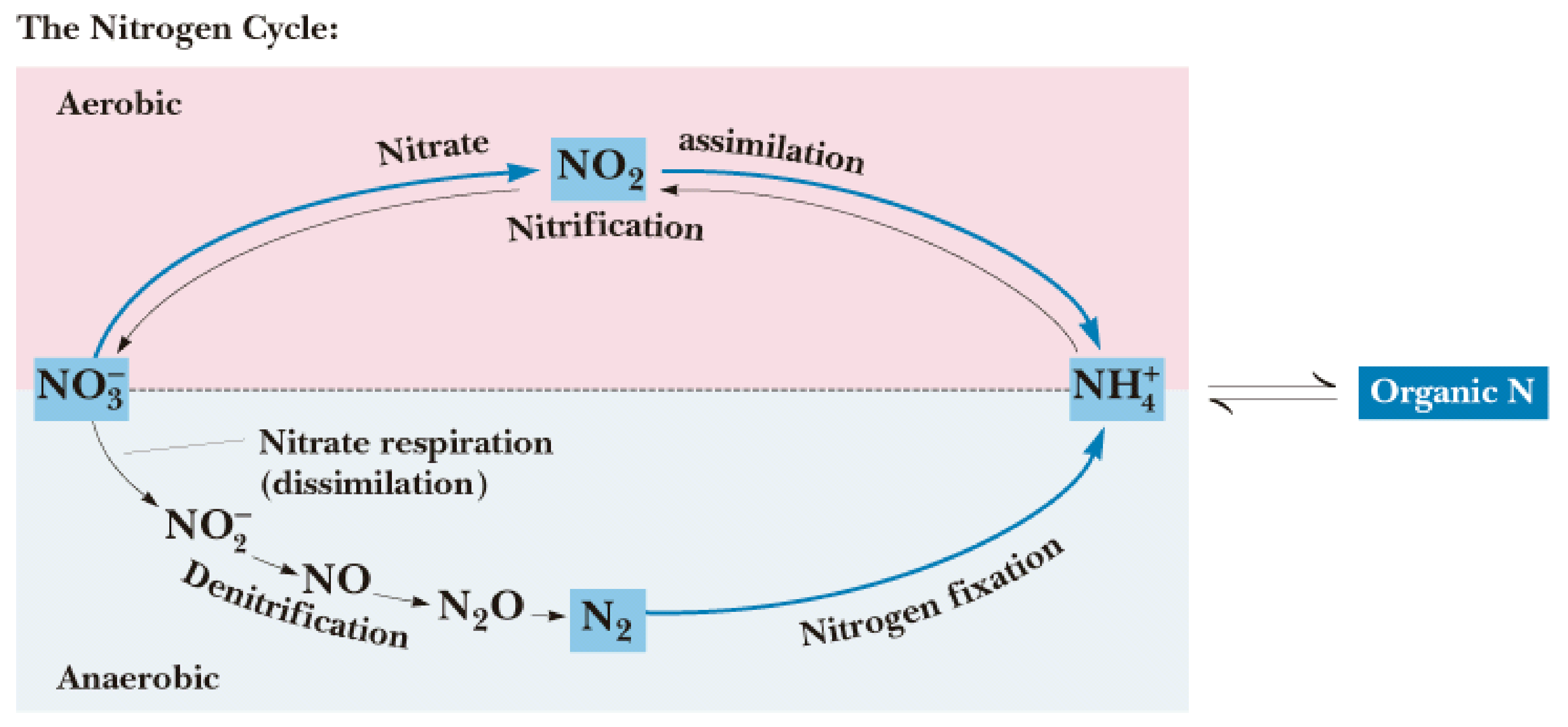


Fig. 5-8 **C_4 Pathway.** The C_4 pathway adds CO_2 to either pyruvate or phosphoenolpyruvate to produce oxaloacetate.

Фиксация азота



Фиксация азота

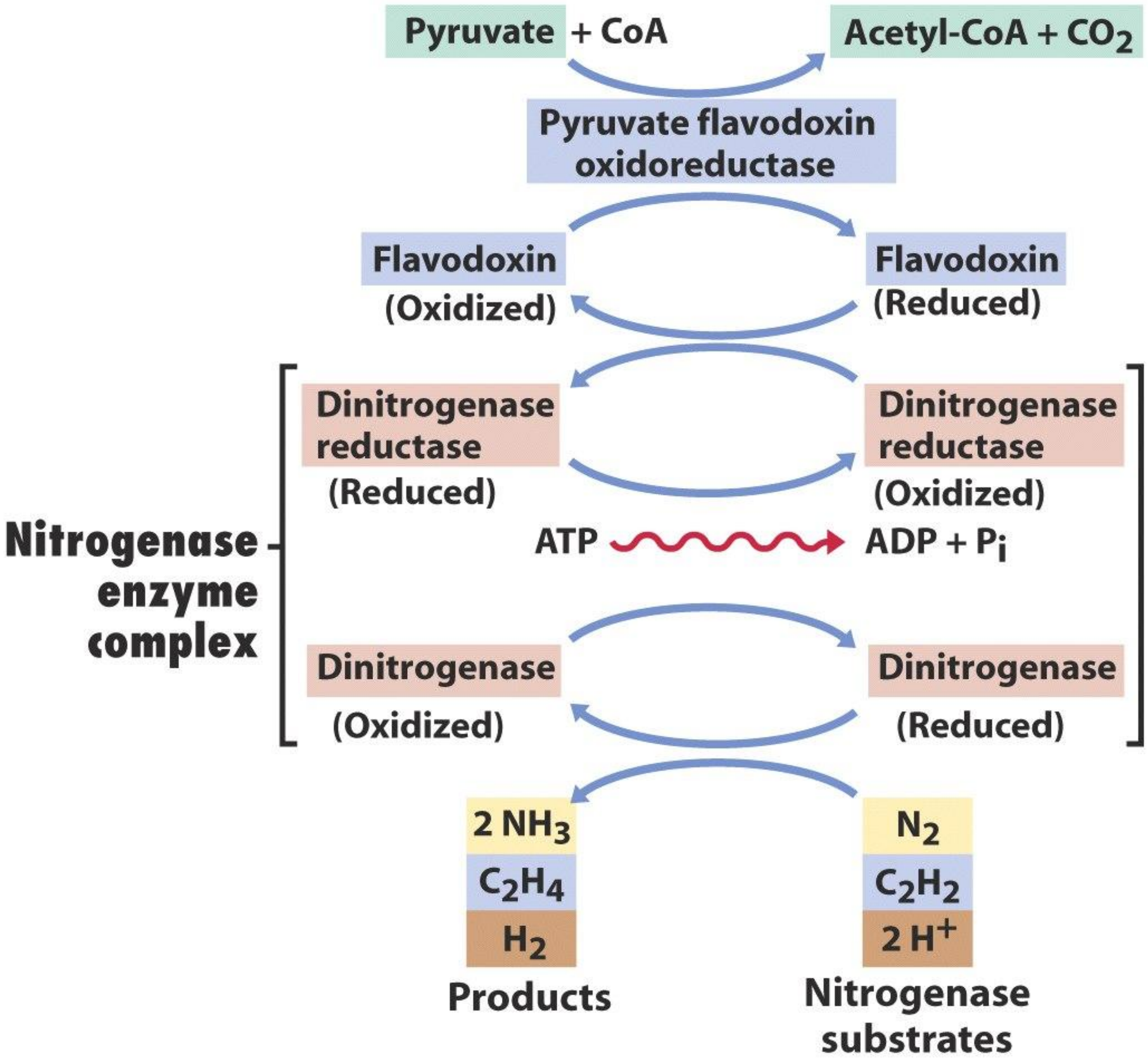


Figure 17-71a Brock Biology of Microorganisms 11/e
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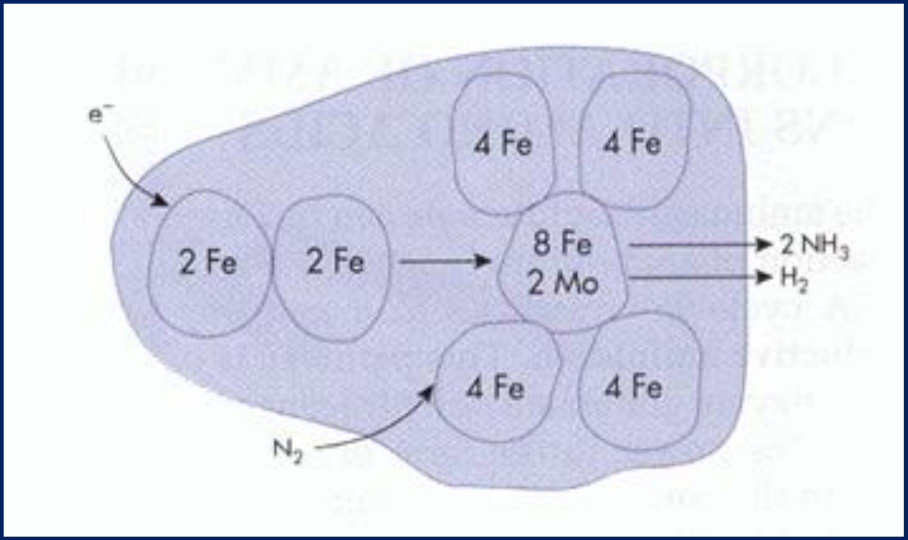
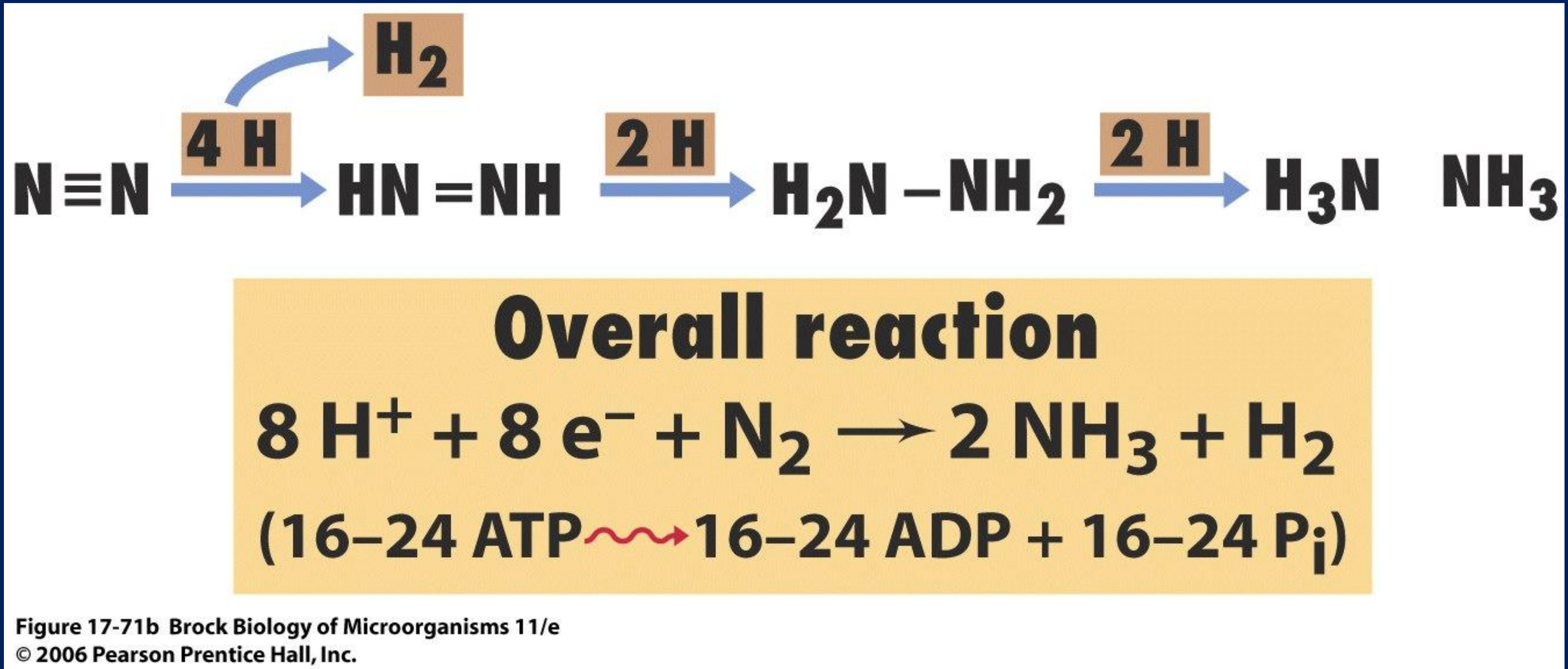
$$8H^+ + 8e^- + N_2 \longrightarrow 2NH_3 + H_2$$

$$16\text{-}24\ ATP \longrightarrow 16\text{-}24ADP + 16\text{-}24Pi$$

A 220 kD heterotetramer



Each molecule of enzyme contains 2 Mo, 32 Fe,
30 equivalents of acid-labile sulfide (FeS clusters, etc)
4 of 4Fe-4S clusters +
2 FeMo-Co, an iron-molybdenum cofactor
Nitrogenase is slow - 12 e^- pairs per second, i.e., only 3 N_2 per second



Overall reaction

$$8\,H^+ + 8\,e^- + N_2 \longrightarrow 2\,NH_3 + H_2$$

$$(16\text{–}24\ \text{ATP} \rightsquigarrow 16\text{–}24\ \text{ADP} + 16\text{–}24\ P_i)$$

Figure 17-71b Brock Biology of Microorganisms 11/e
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Some nitrogen-fixing organisms

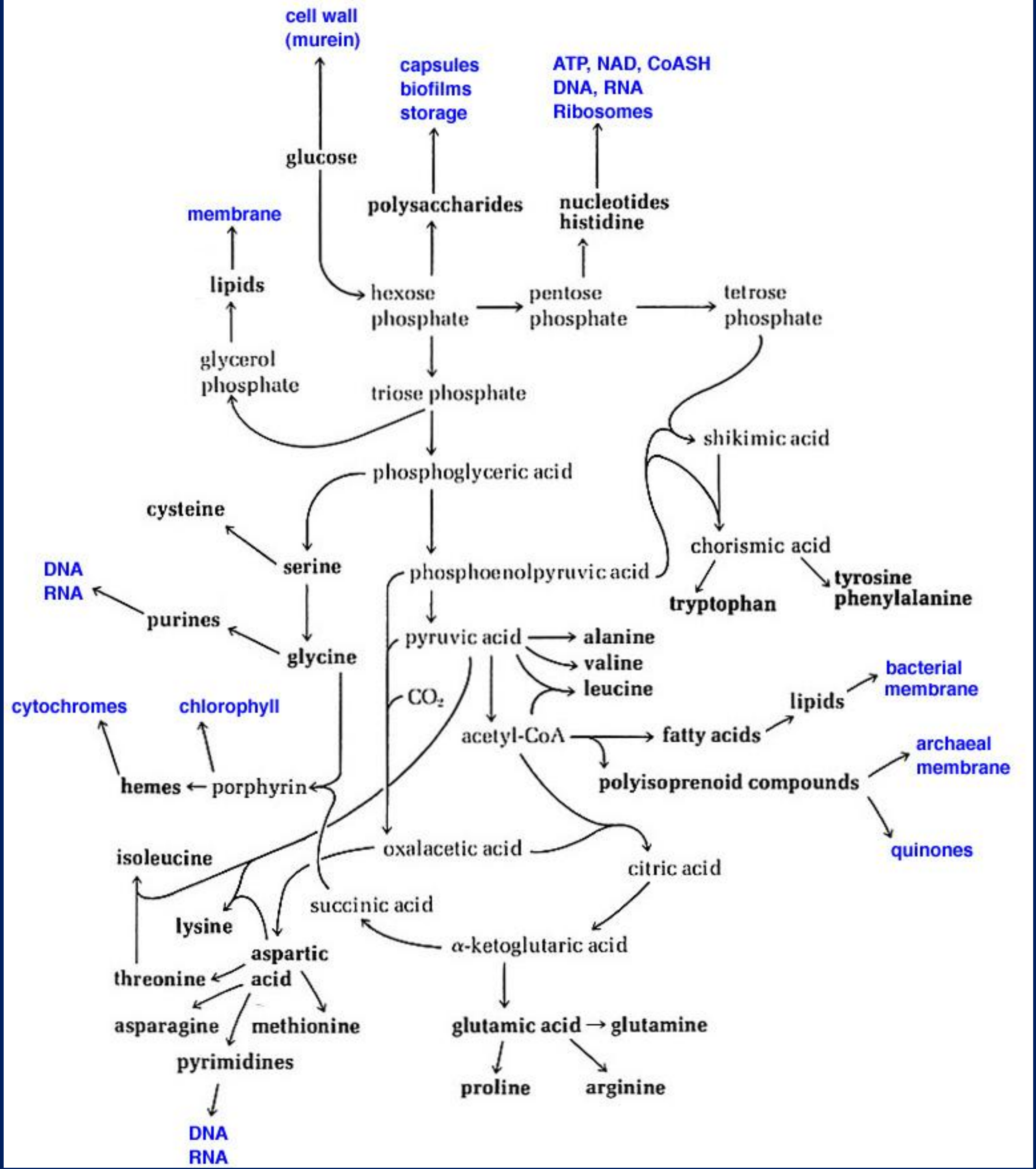
Chemo-organotrophs	**phototrophs**	**Chemo-lithotrophs**
	Free-living aerobes	
Azotobacter spp.	*Cyanobacteria*	*Alcaligenes*
Azomonas	(various,but not all)	*Thiobacillus*
Beijerinckia		
Bacillus polymyxa		
	Free-living anaerobes	
Bacteria:	Bacteria:	Archaea:
Clostridium spp.	*Chlorobium*	*Methanosarcina*
Desulfovibrio	*Rhodospirillum*	*Methanoccous*
Desulfotomacullum		

N2 fixation occurs only under anoxic condition

Anabolic Pathways

- Anabolic reactions are **synthesis reactions – require energy and source of metabolites**
- Energy derived from ATP from catabolic reactions
- Glycolysis, the Krebs cycle, and the pentose phosphate pathway provide twelve basic precursor metabolites from which all macromolecules and cellular structures are made
- Many anabolic pathways are the reversal of the catabolic pathways
  - Reactions that can proceed in either direction are amphibolic

Metabolic pathways in bacteria



cell wall (murein)

capsules biofilms storage

ATP, NAD, CoASH DNA, RNA Ribosomes

glucose

polysaccharides

nucleotides histidine

membrane

lipids

hexose phosphate → pentose phosphate → tetrose phosphate

glycerol phosphate

triose phosphate

shikimic acid

phosphoglyceric acid

cysteine

serine

chorismic acid

tryptophan

tyrosine phenylalanine

DNA RNA

purines

glycine

phosphoenolpyruvic acid

pyruvic acid → alanine

valine

leucine

CO$_2$

bacterial membrane

lipids

cytochromes

chlorophyll

acetyl-CoA → fatty acids

archaeal membrane

hemes ← porphyrin

polyisoprenoid compounds

quinones

isoleucine

oxalacetic acid

citric acid

succinic acid

lysine

aspartic acid

α-ketoglutaric acid

threonine

asparagine

methionine

glutamic acid → glutamine

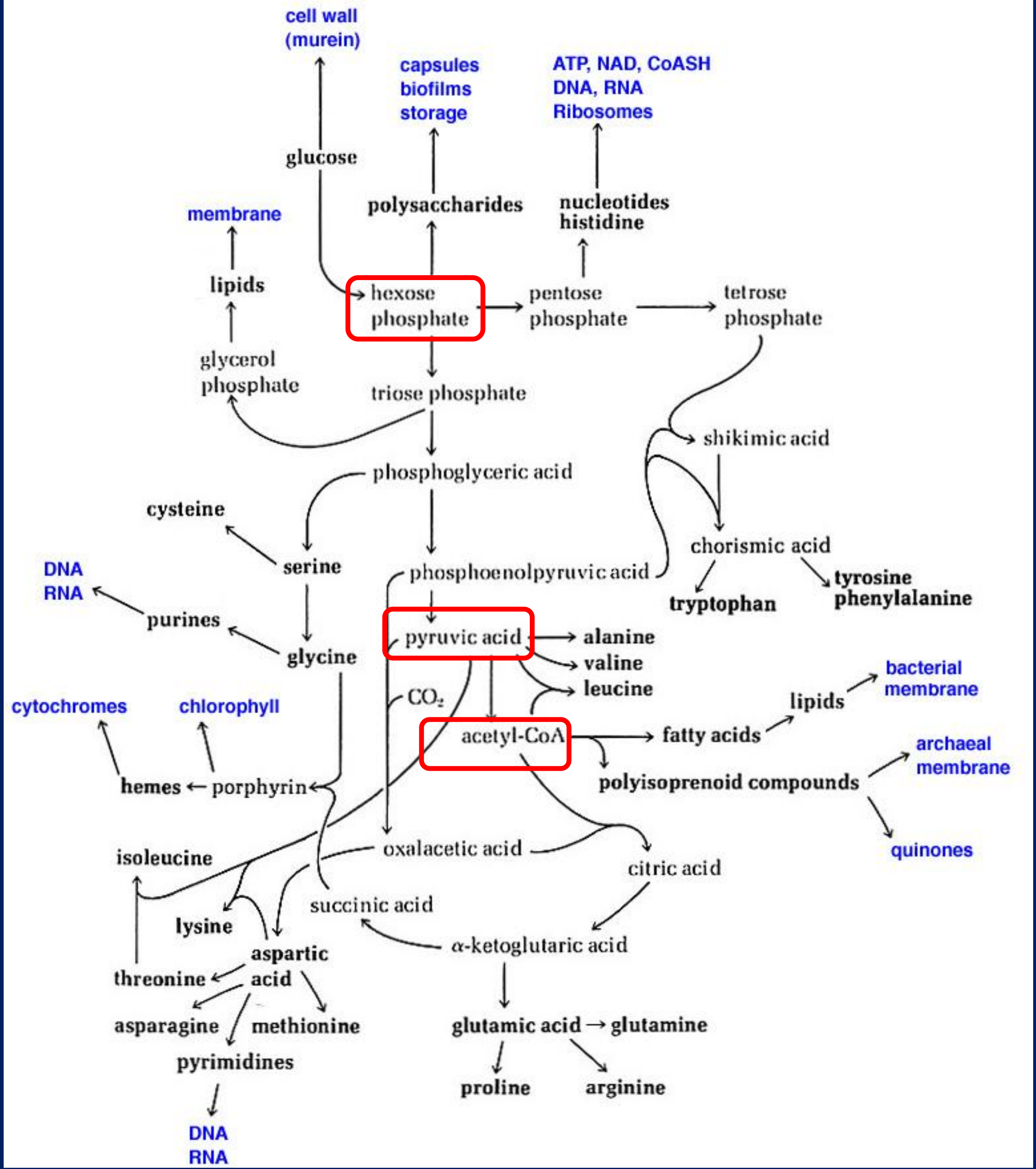
pyrimidines

proline

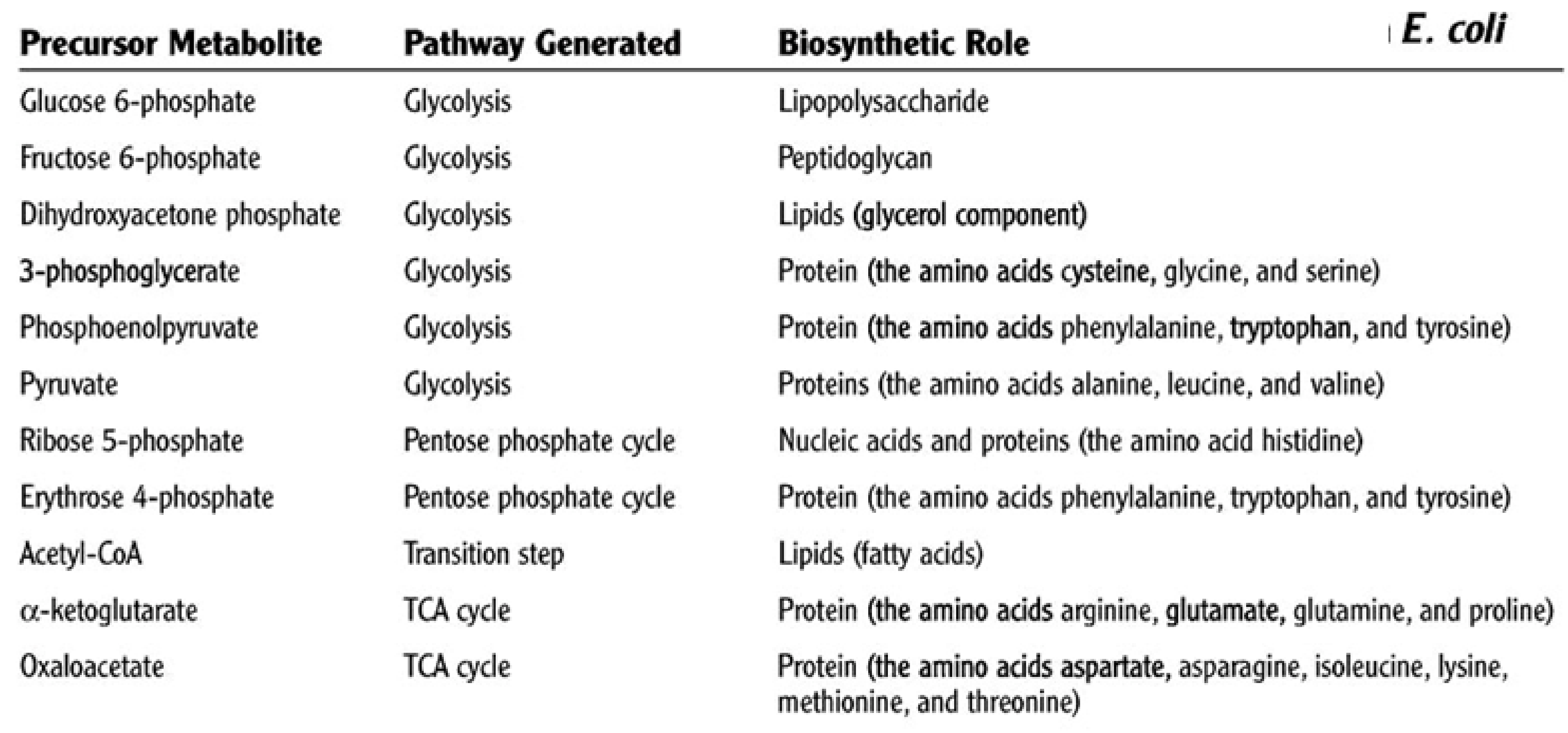
arginine

DNA RNA

Main metabolites in bacteria



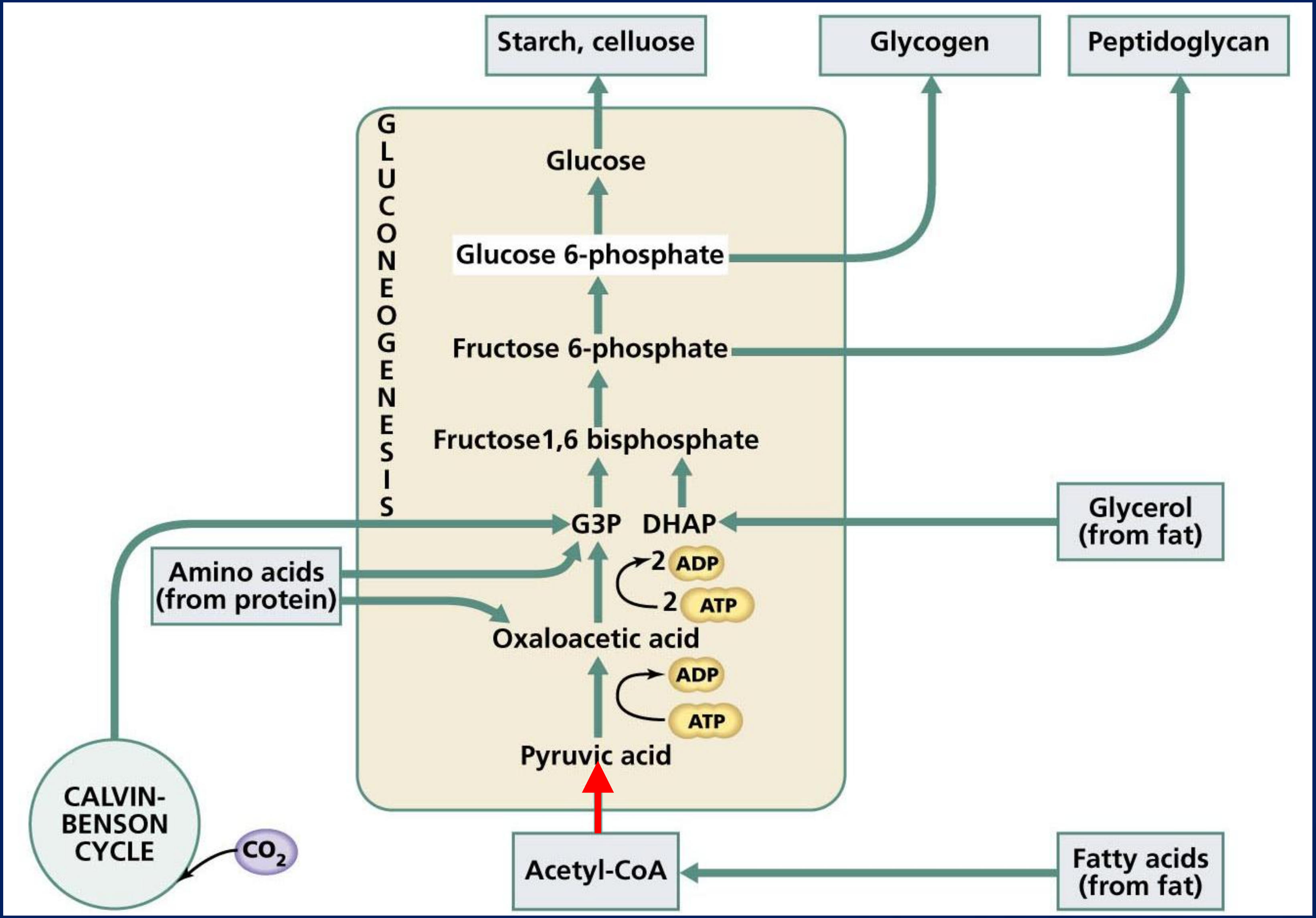
Ключевые метаболиты в биосинтезах



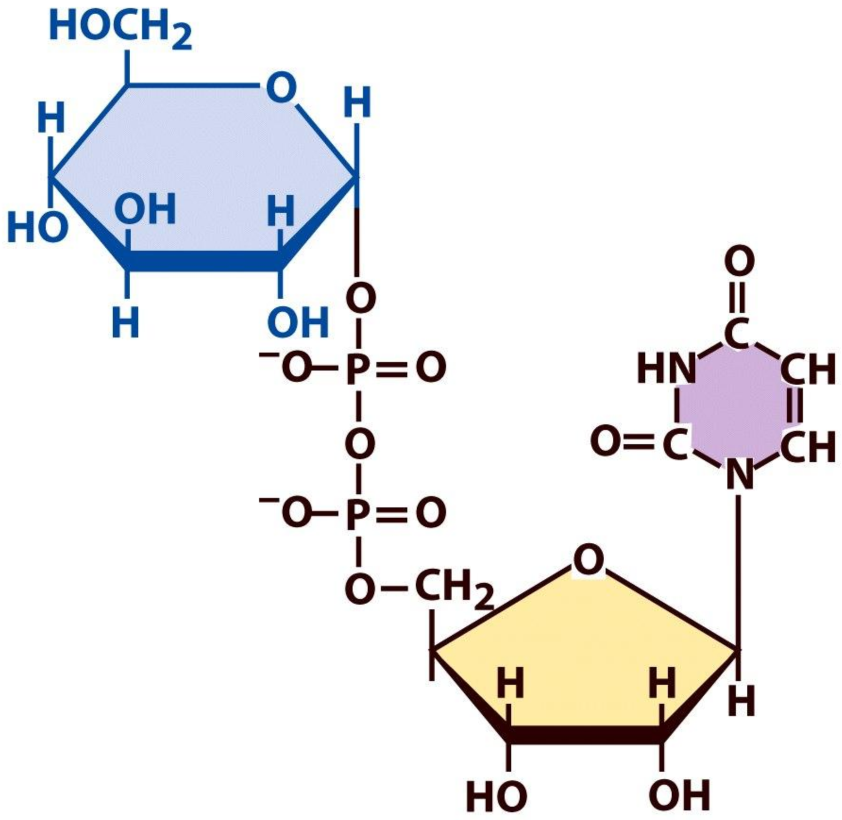
E. coli

Precursor Metabolite	Pathway Generated	Biosynthetic Role
Glucose 6-phosphate	Glycolysis	Lipopolysaccharide
Fructose 6-phosphate	Glycolysis	Peptidoglycan
Dihydroxyacetone phosphate	Glycolysis	Lipids (glycerol component)
3-phosphoglycerate	Glycolysis	Protein (the amino acids cysteine, glycine, and serine)
Phosphoenolpyruvate	Glycolysis	Protein (the amino acids phenylalanine, tryptophan, and tyrosine)
Pyruvate	Glycolysis	Proteins (the amino acids alanine, leucine, and valine)
Ribose 5-phosphate	Pentose phosphate cycle	Nucleic acids and proteins (the amino acid histidine)
Erythrose 4-phosphate	Pentose phosphate cycle	Protein (the amino acids phenylalanine, tryptophan, and tyrosine)
Acetyl-CoA	Transition step	Lipids (fatty acids)
α-ketoglutarate	TCA cycle	Protein (the amino acids arginine, glutamate, glutamine, and proline)
Oxaloacetate	TCA cycle	Protein (the amino acids aspartate, asparagine, isoleucine, lysine, methionine, and threonine)

Anabolic Pathways – Carbohydrate Biosynthesis



❑ **Polysaccharides** are synthesized from activated forms of hexoses such as UDPG.



Uridine diphosphoglucose (UDPG)

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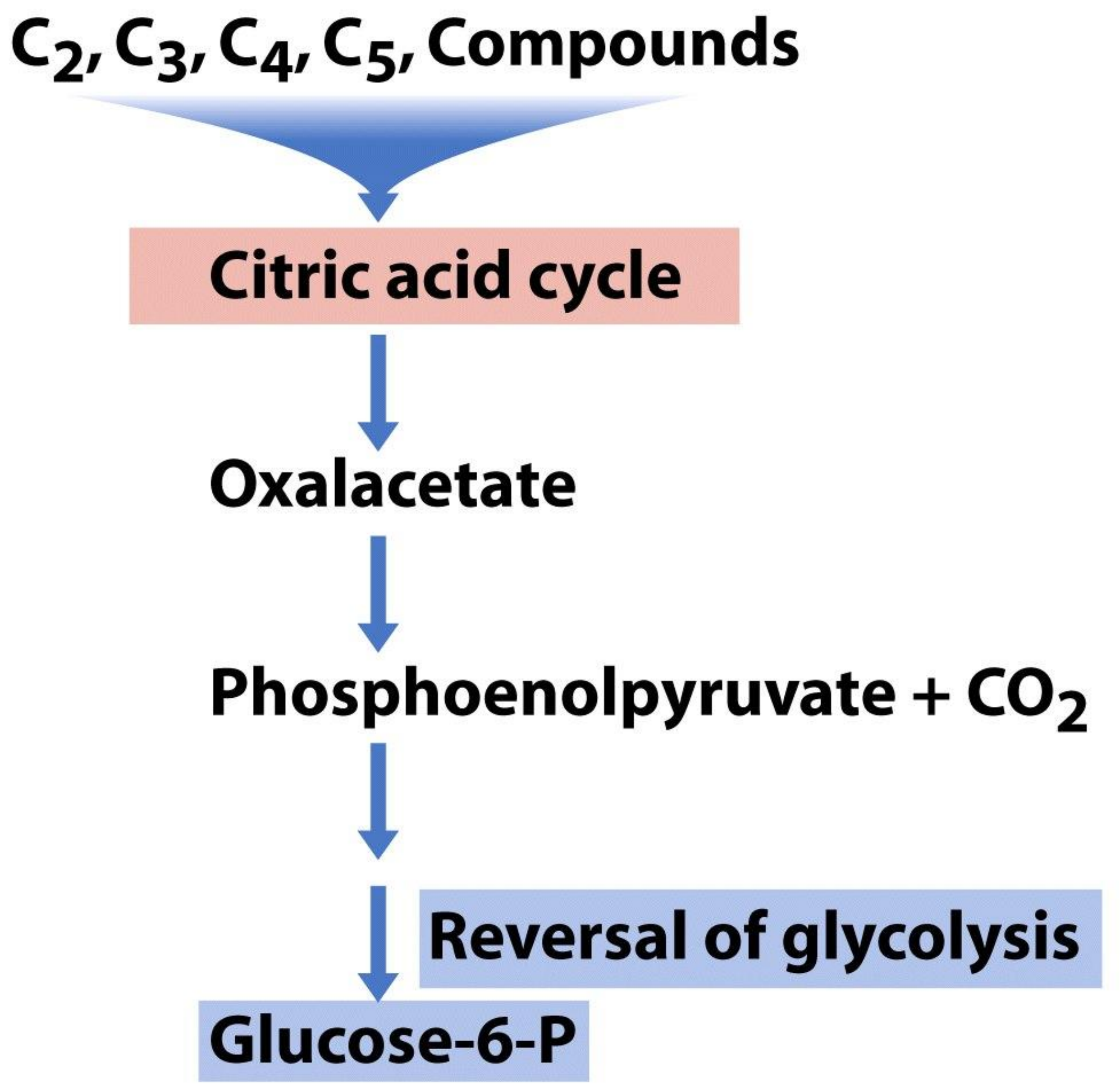
Glycogen is biosynthesized from *adenosine*-phosphoglucose by the sequential addition of glucose

Gluconeogenesis



C_2, C_3, C_4, C_5, Compounds

Citric acid cycle

Oxalacetate

Phosphoenolpyruvate + CO_2

Reversal of glycolysis

Glucose-6-P

Figure 5-25c Brock Biology of Microorganisms 11/e
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Pentoses for nucleic acid synthesis are formed by decarboxylation of hexoses like glucose-6-phosphate

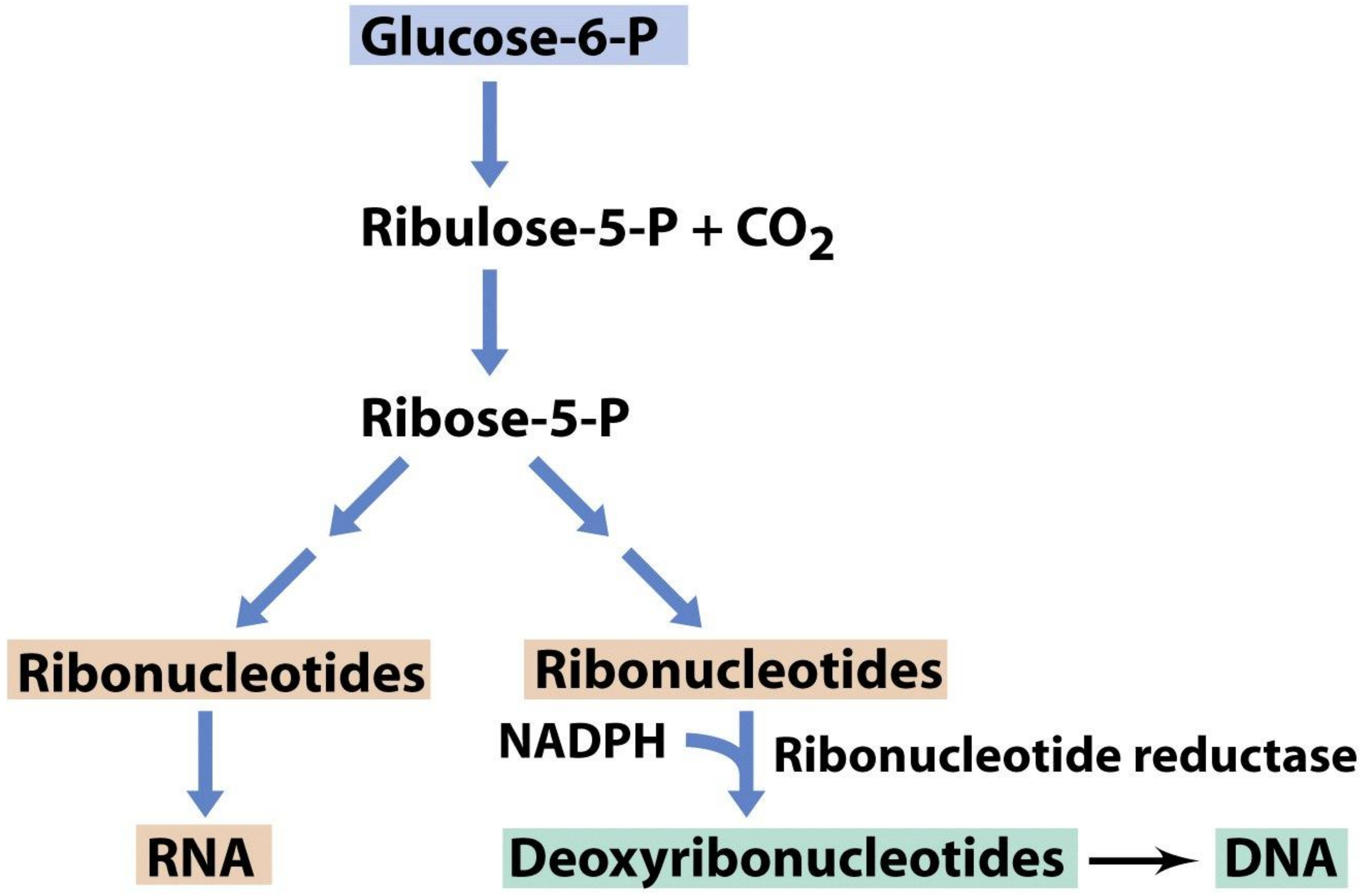

Figure 5-25d Brock Biology of Microorganisms 11/e
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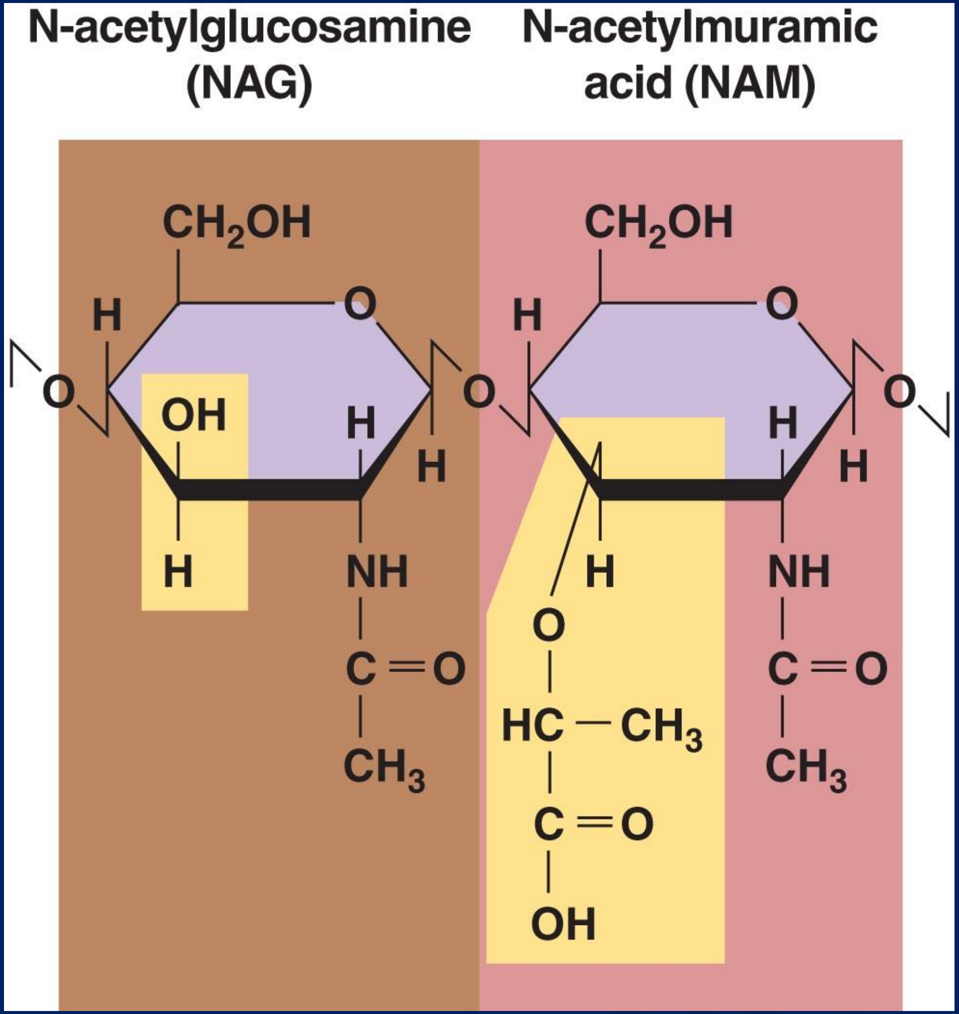
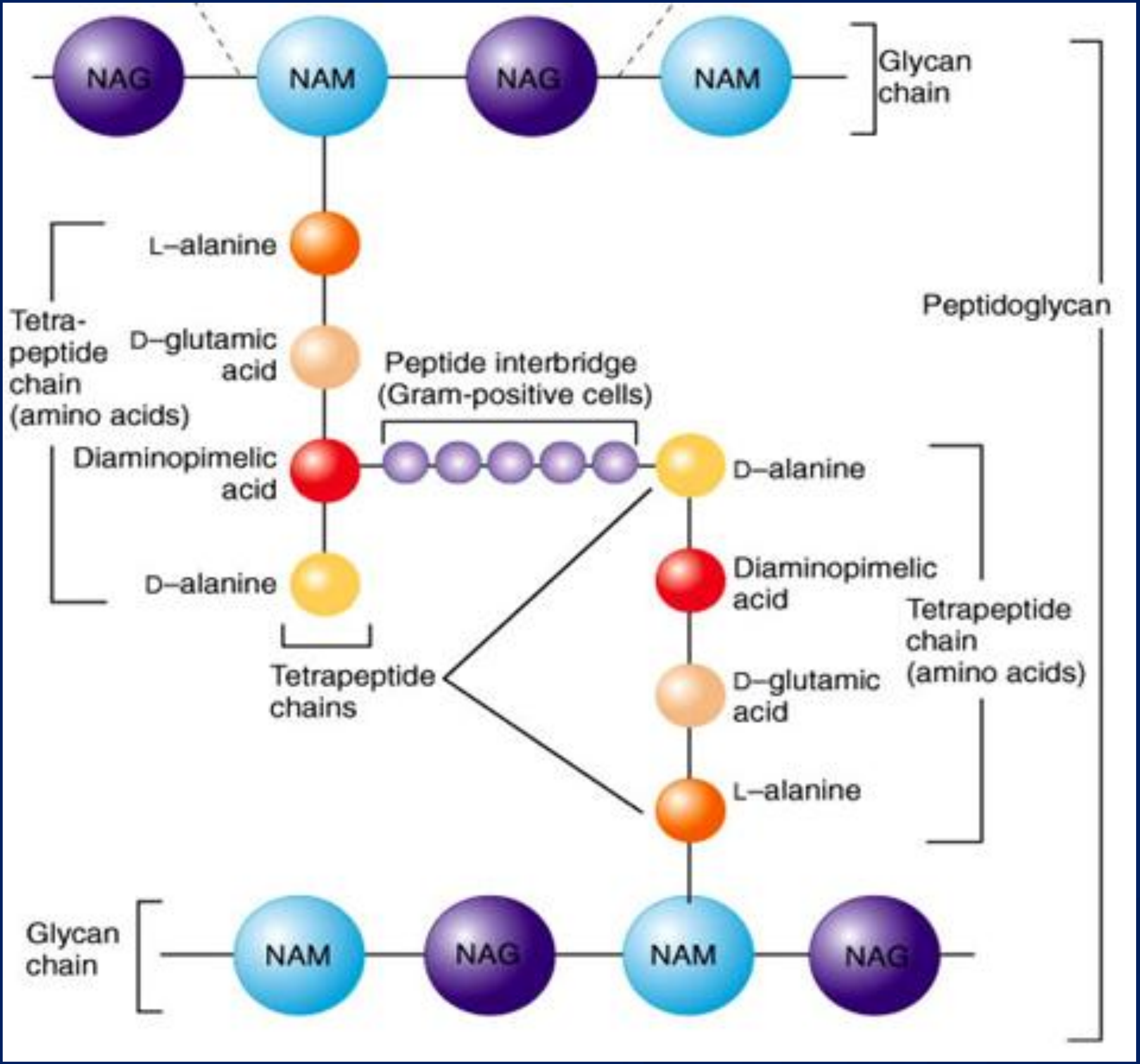
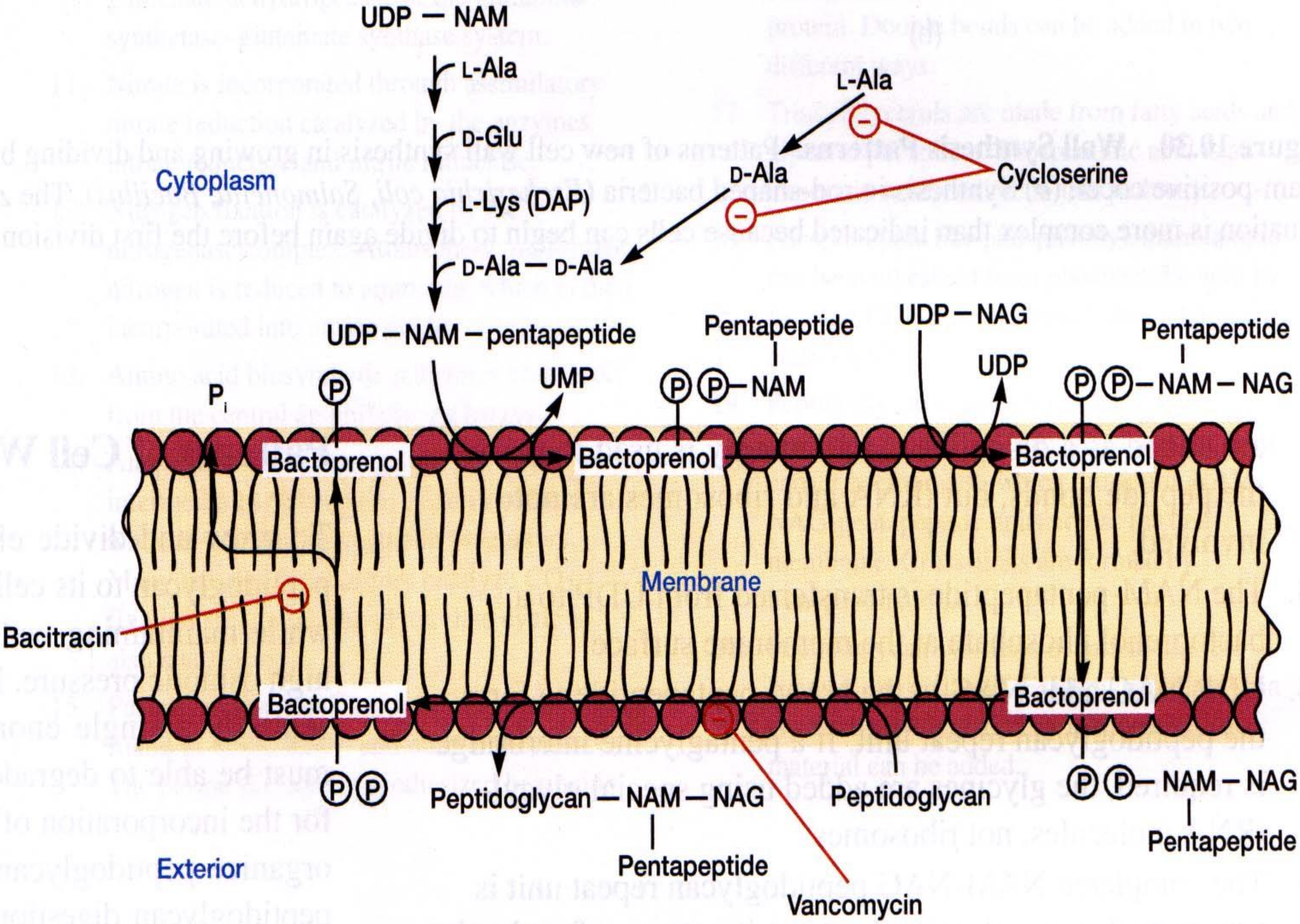
- **Peptidoglycan synthesis**: synthesis of polymer from N-acetyl-muramic acid and transpeptidation

- Polymer of disaccharide:
  - N-acetylglucosamine (**NAG**)
  - N-acetylmuramic acid (**NAM**)

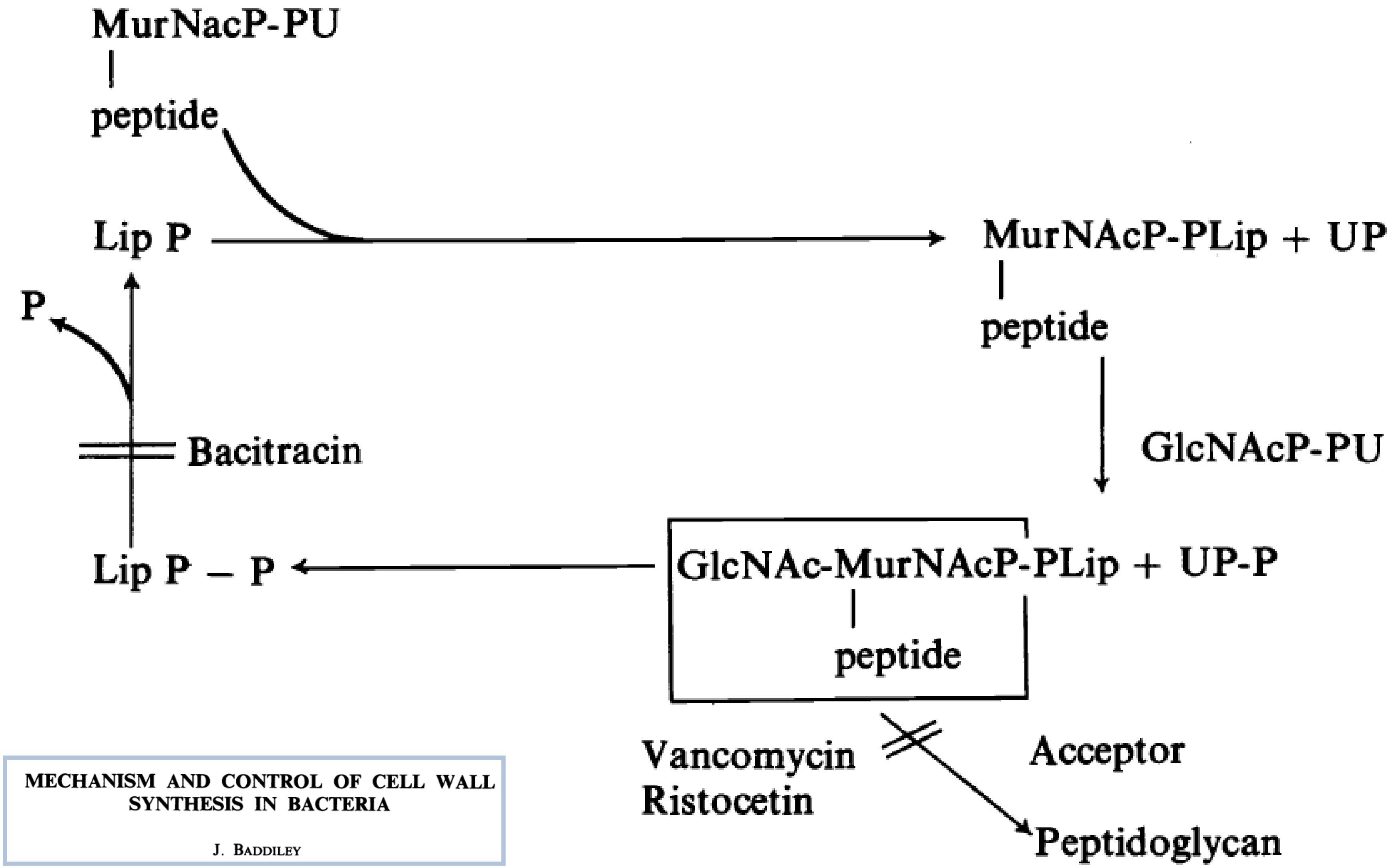


Peptidoglycan


NAG
NAM
NAG
NAM
Glycan chain
Peptidoglycan
L–alanine
Tetra-peptide chain (amino acids)
D–glutamic acid
Peptide interbridge (Gram-positive cells)
Diaminopimelic acid
D–alanine
D–alanine
Diaminopimelic acid
Tetrapeptide chain (amino acids)
Tetrapeptide chains
D–glutamic acid
L–alanine
Glycan chain
NAM
NAG
NAM
NAG


UDP — NAM
L-Ala
D-Glu
L-Lys (DAP)
D-Ala — D-Ala
UDP — NAM — pentapeptide
L-Ala
D-Ala
Cycloserine
Cytoplasm
Pentapeptide
P P — NAM
UDP — NAG
UDP
UMP
Pentapeptide
P P — NAM — NAG
P_i
P
Bactoprenol
Bactoprenol
Bactoprenol
Membrane
Bacitracin
Bactoprenol
Bactoprenol
P P
Peptidoglycan — NAM — NAG
Pentapeptide
Peptidoglycan
P P — NAM — NAG
Pentapeptide
Vancomycin
Exterior

CELL WALL SYNTHESIS IN BACTERIA



MurNacP-PU
peptide

Lip P → MurNAcP-PLip + UP
peptide

GlcNAcP-PU

GlcNAc-MurNAcP-PLip + UP-P
peptide

Lip P – P

P

Bacitracin

Vancomycin
Ristocetin

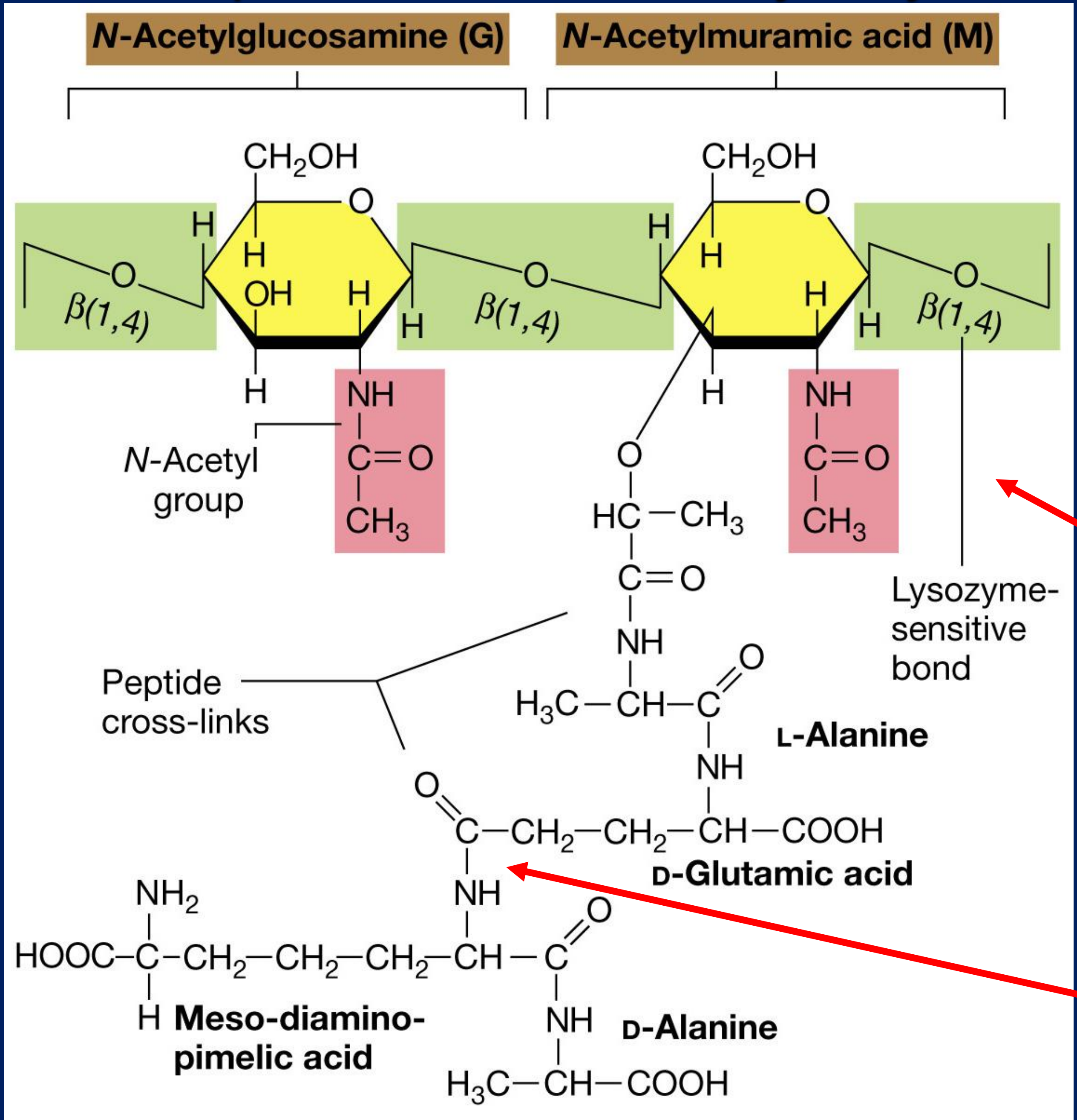
Acceptor

Peptidoglycan

MECHANISM AND CONTROL OF CELL WALL SYNTHESIS IN BACTERIA

J. Baddiley

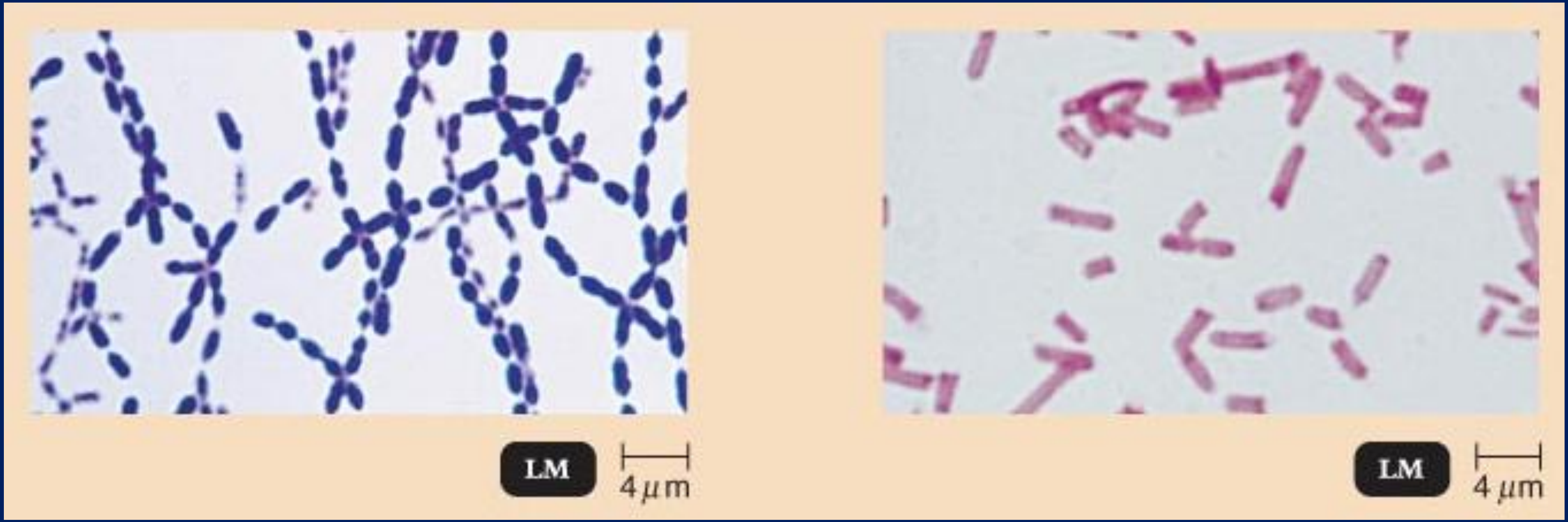
Compare the effects of lysozyme and penicillin



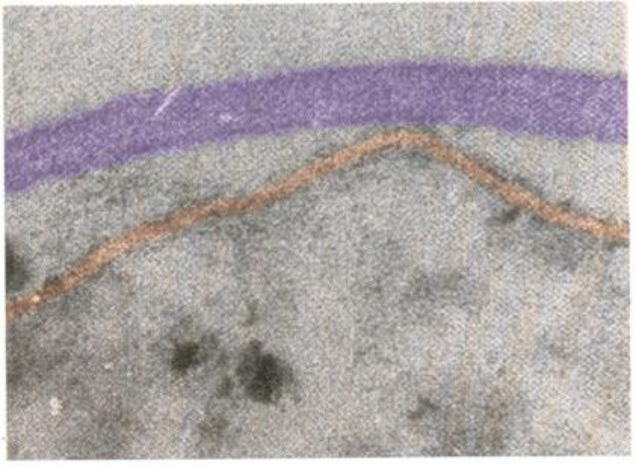
Lysozyme

Penicillin-prevents crosslinking

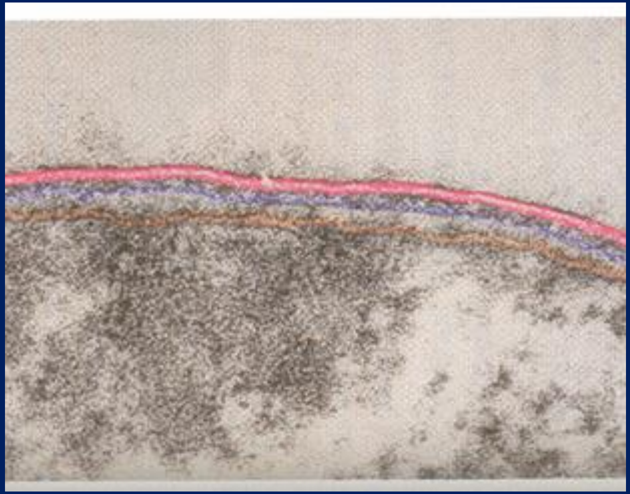
The Gram Stain



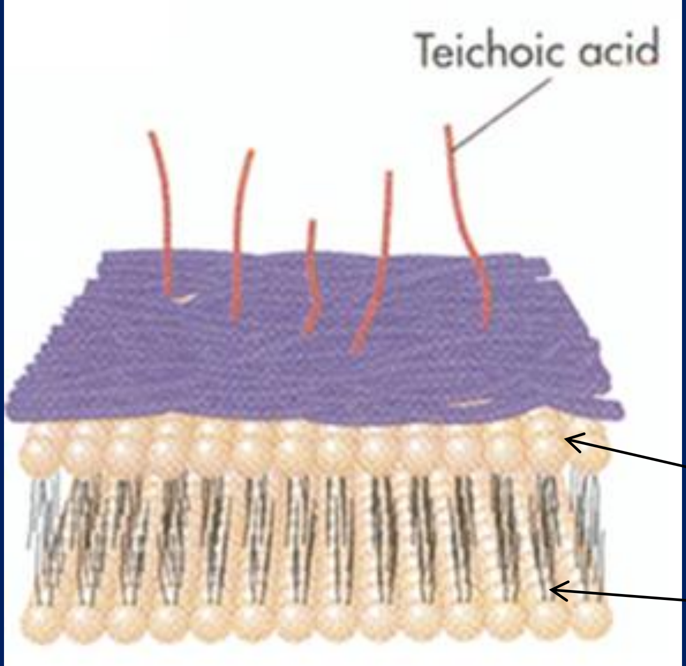
(a) Gram-Positive **(b) Gram-Negative**

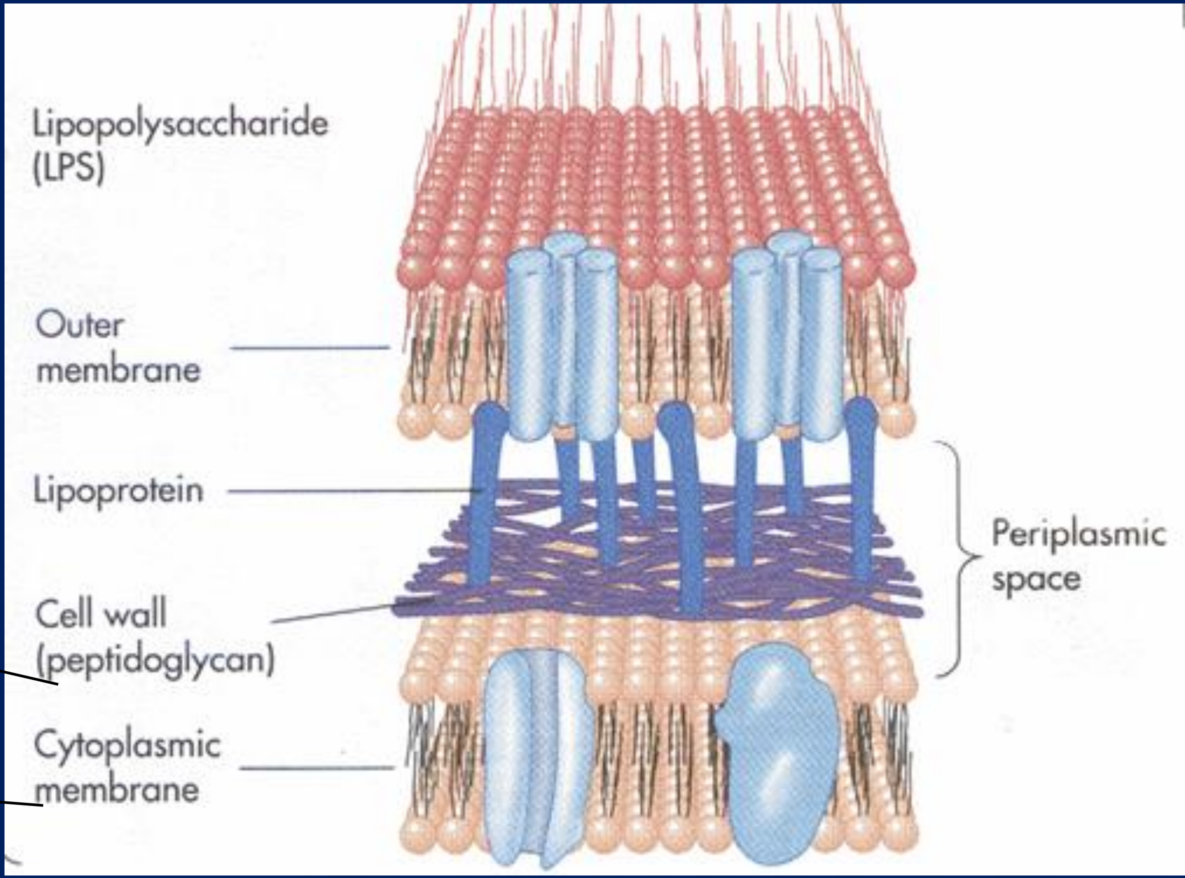


Грам-

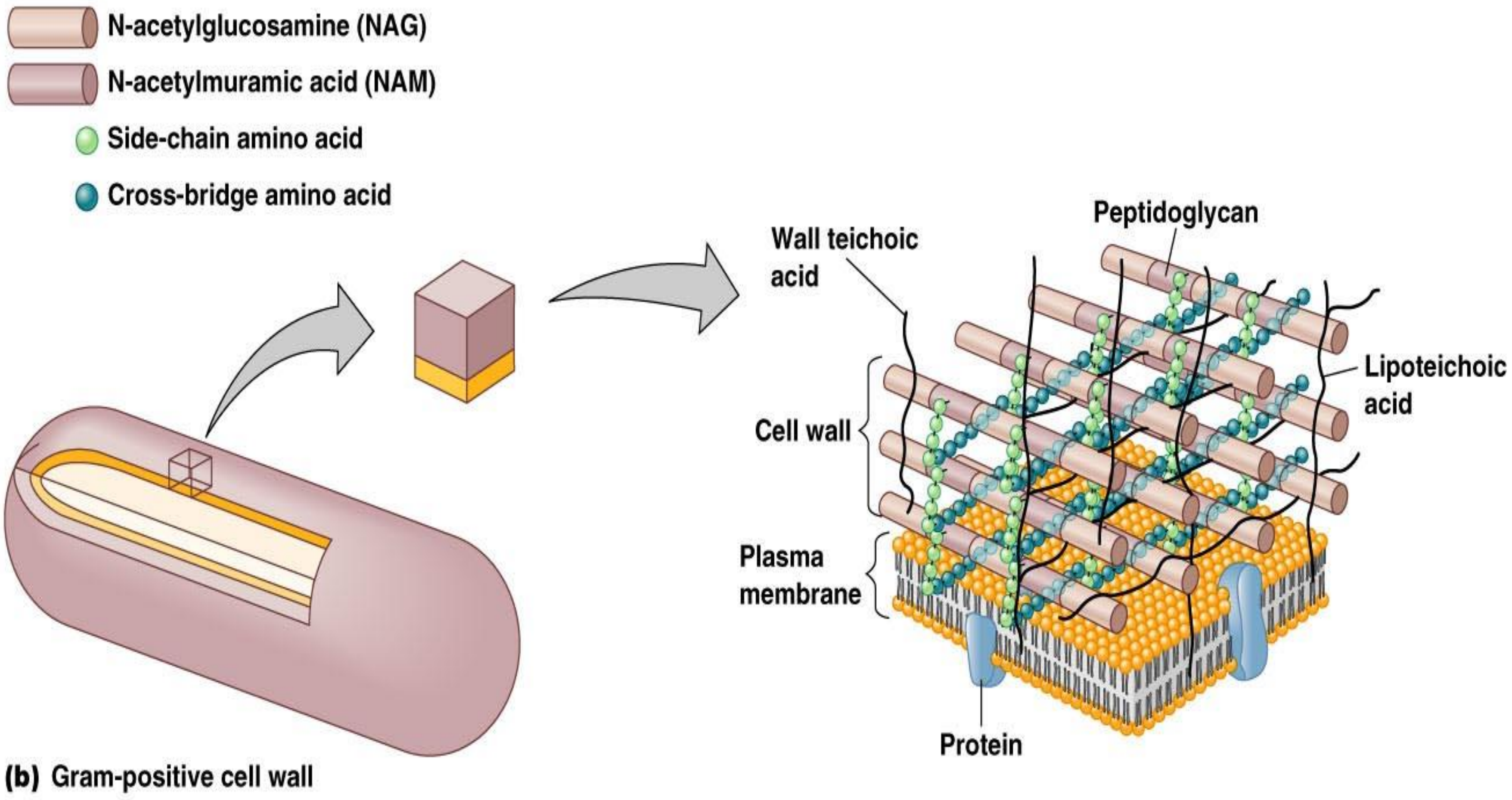


Грам+


Teichoic acid


Lipopolysaccharide (LPS)
Outer membrane
Lipoprotein
Cell wall (peptidoglycan)
Cytoplasmic membrane
Periplasmic space

Клеточная стенка Грам+ бактерий



(b) Gram-positive cell wall

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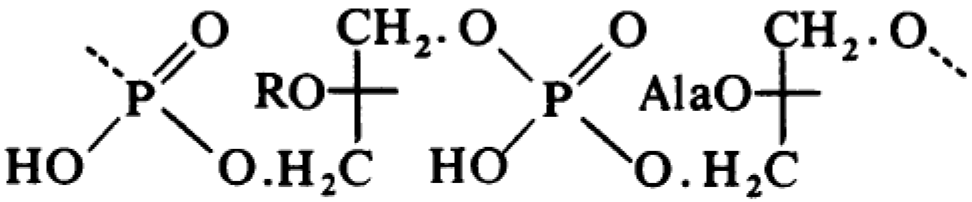
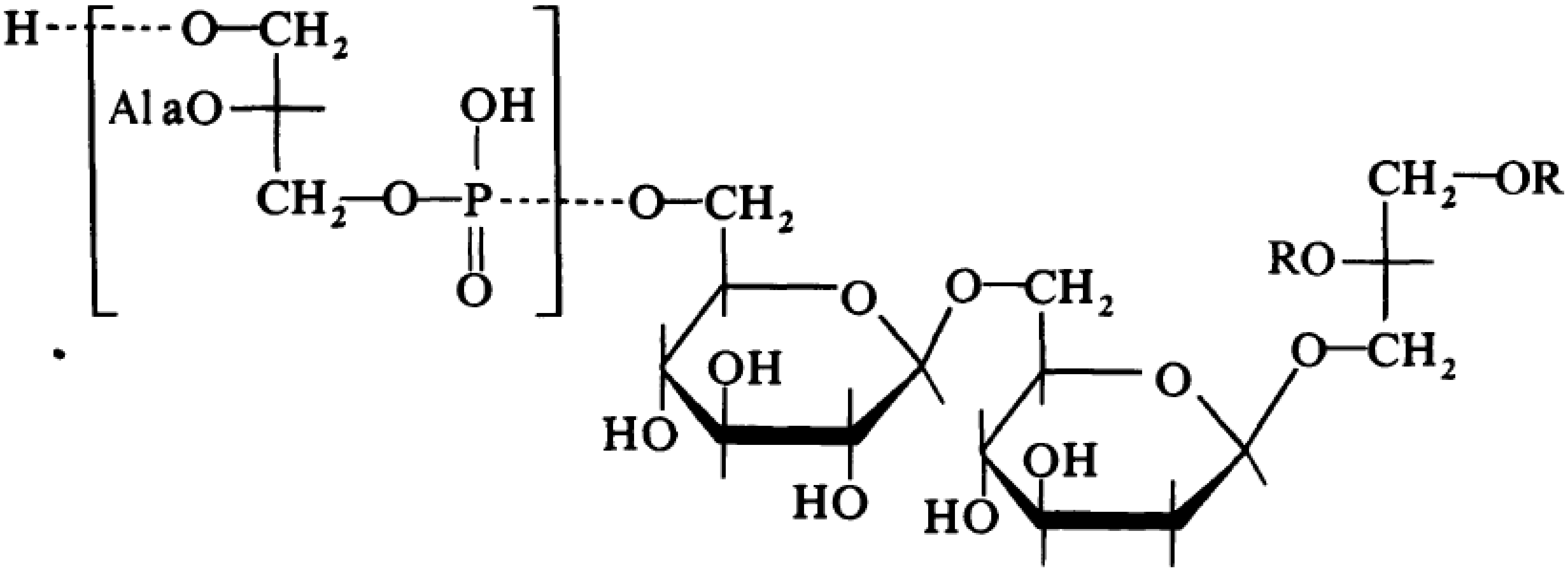
Тейхоевые кислоты



R = glycosyl

Glycerol teichoic acid



Lipoteichoic acid from membrane of *Staphylococcus aureus*

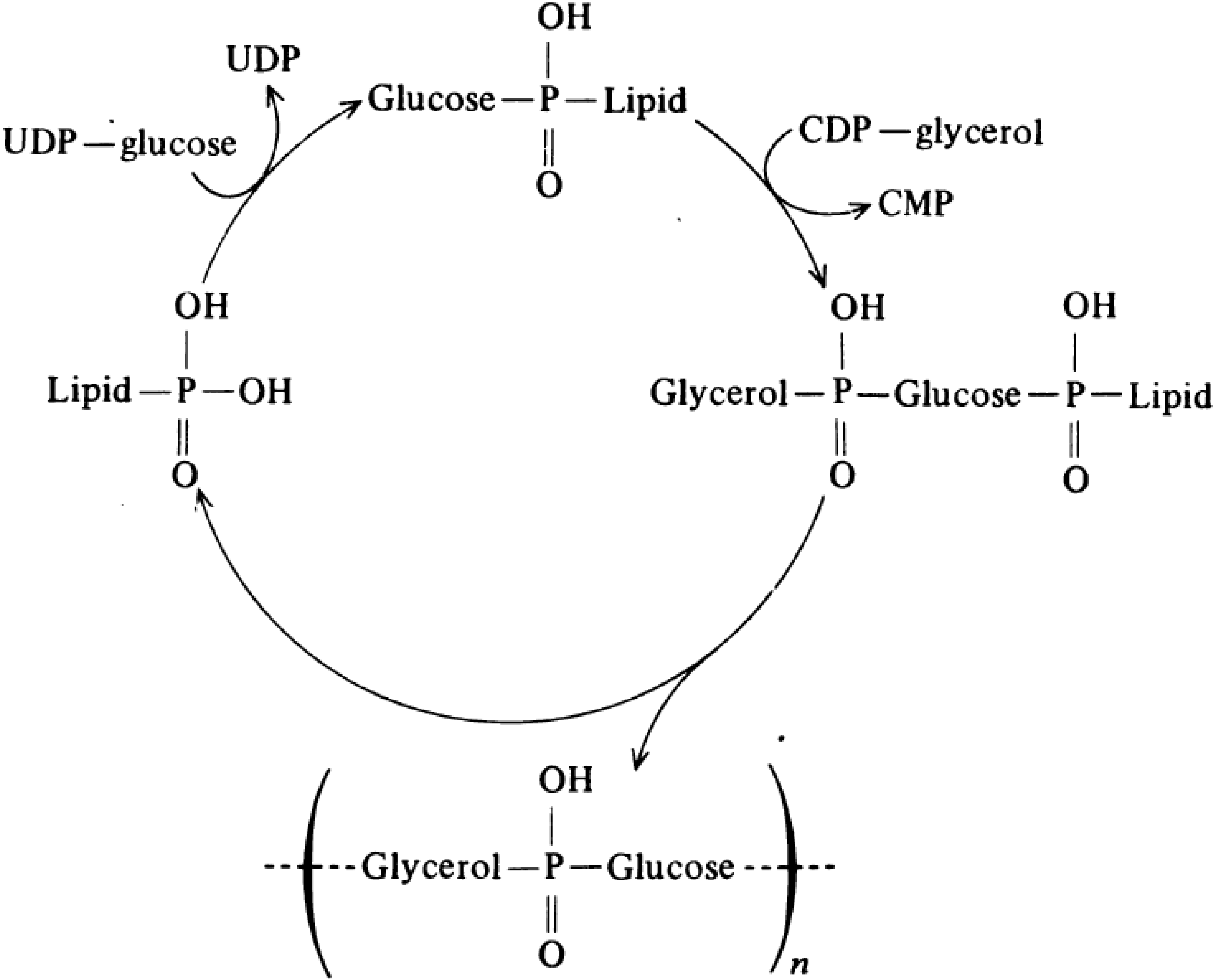
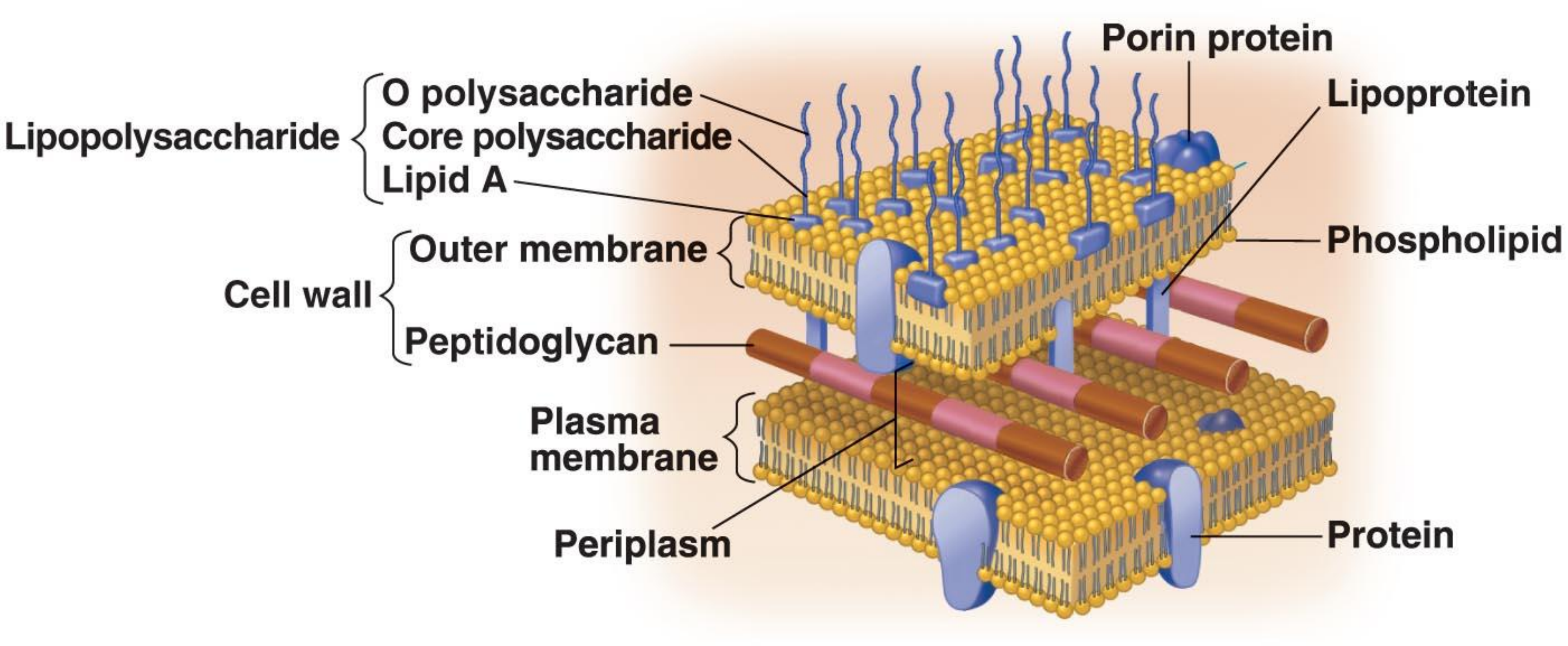
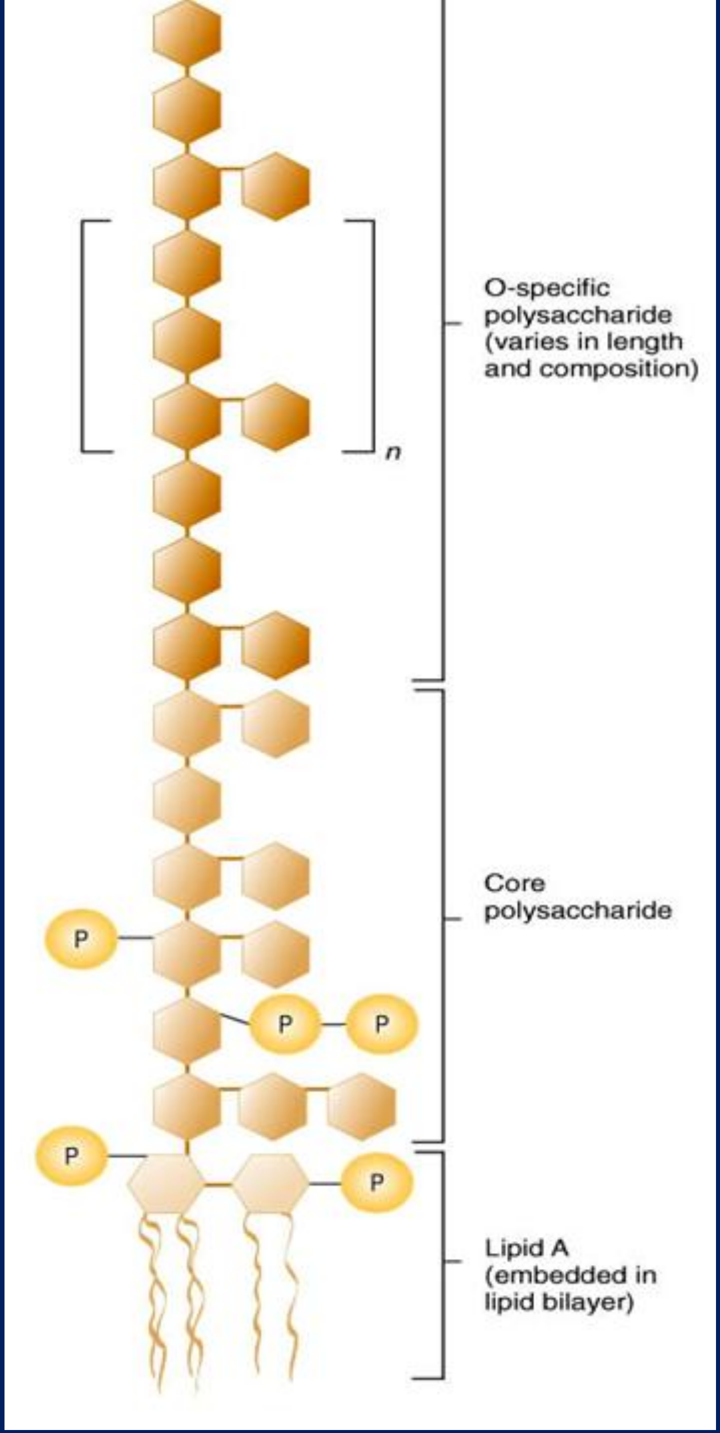


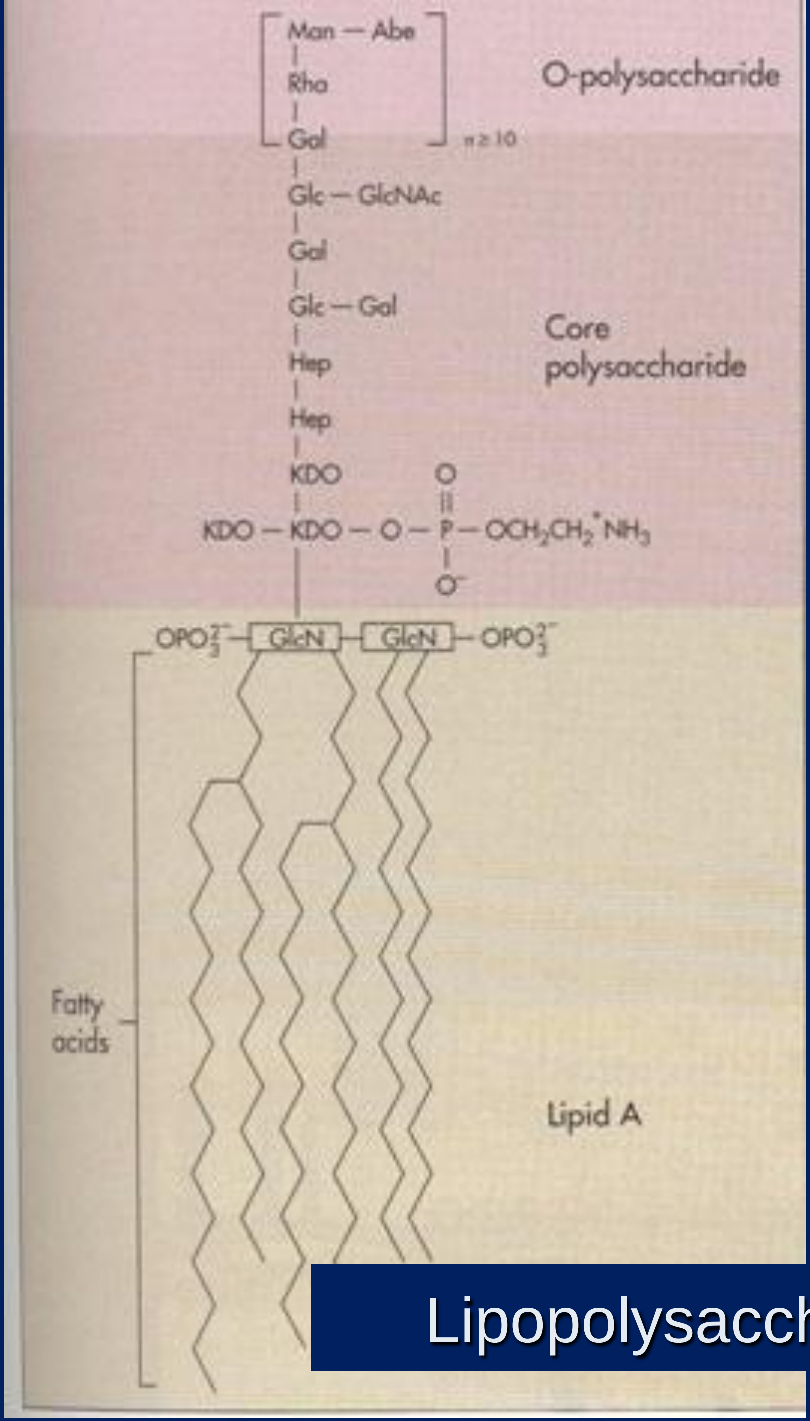
Figure 12. Synthesis of wall teichoic acid in *Bacillus licheniformis*

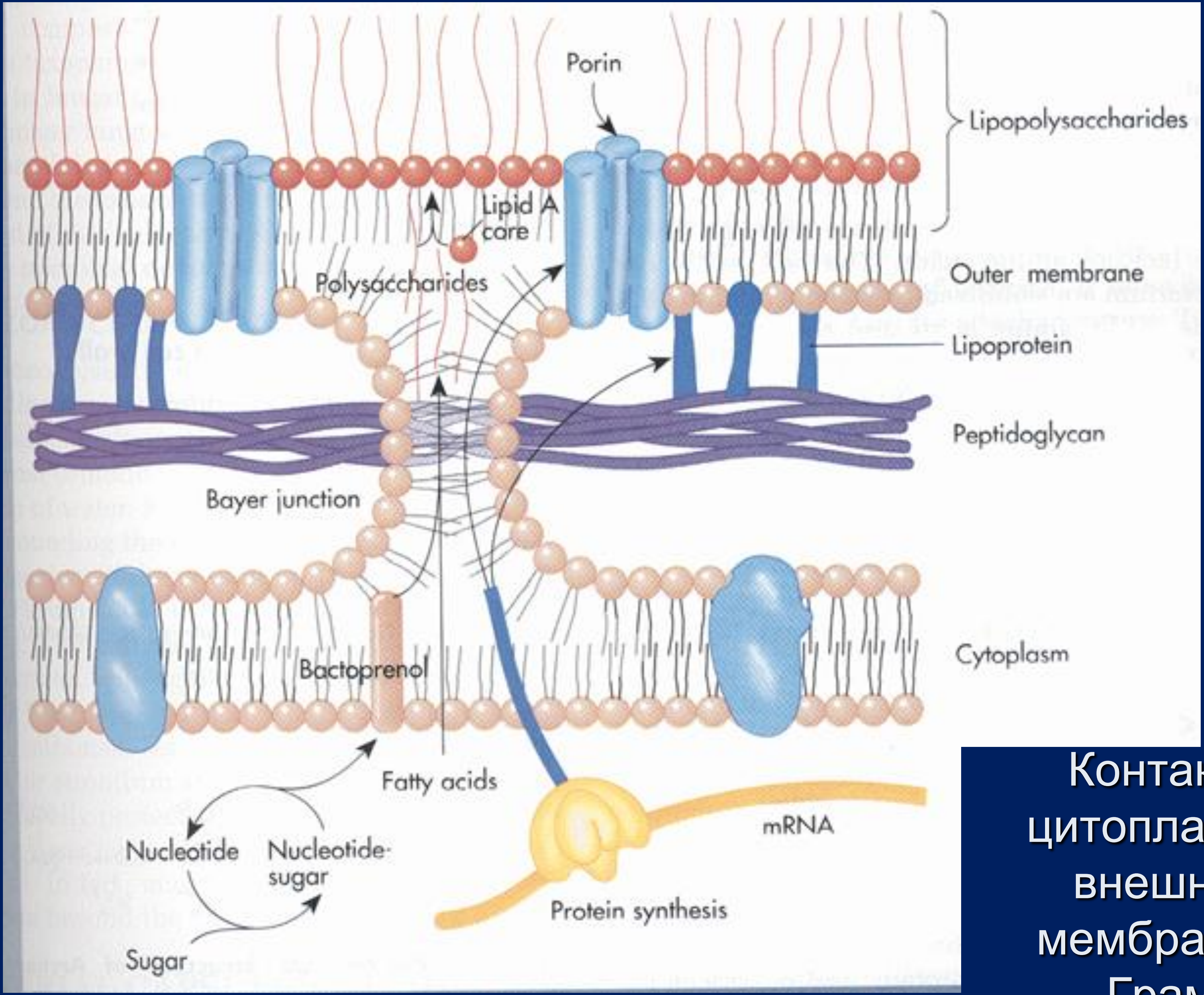
Клеточная стенка Грам- бактерий



Lipopolysaccharide

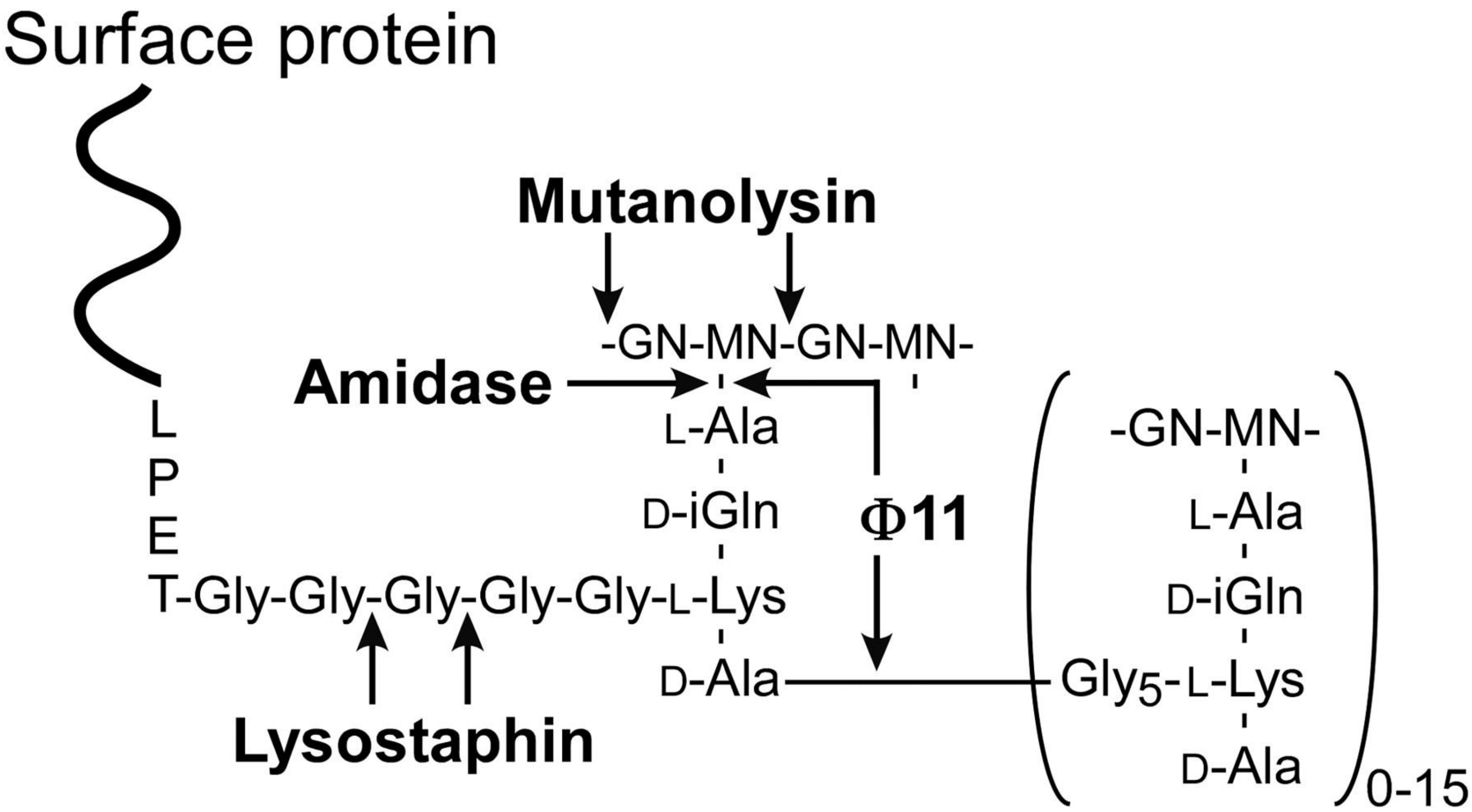

O-specific polysaccharide (varies in length and composition)
n
Core polysaccharide
P
P
P
P
P
Lipid A (embedded in lipid bilayer)





Контакты цитоплазм. и внешней мембраны у Грам-

Cell wall anchor structure of staphylococcal surface proteins.



Marraffini L A et al. Microbiol. Mol. Biol. Rev. 2006;70:192-221

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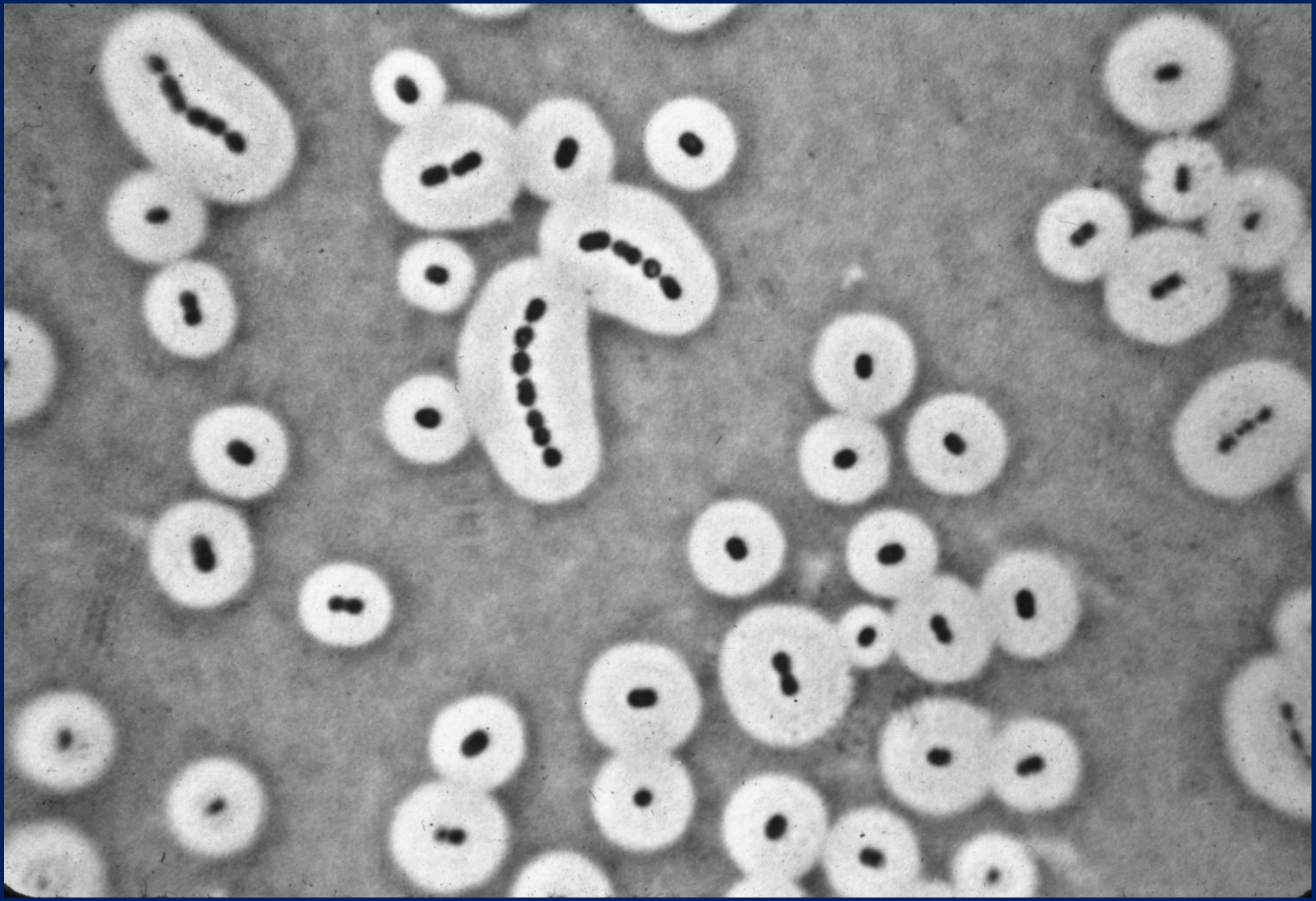
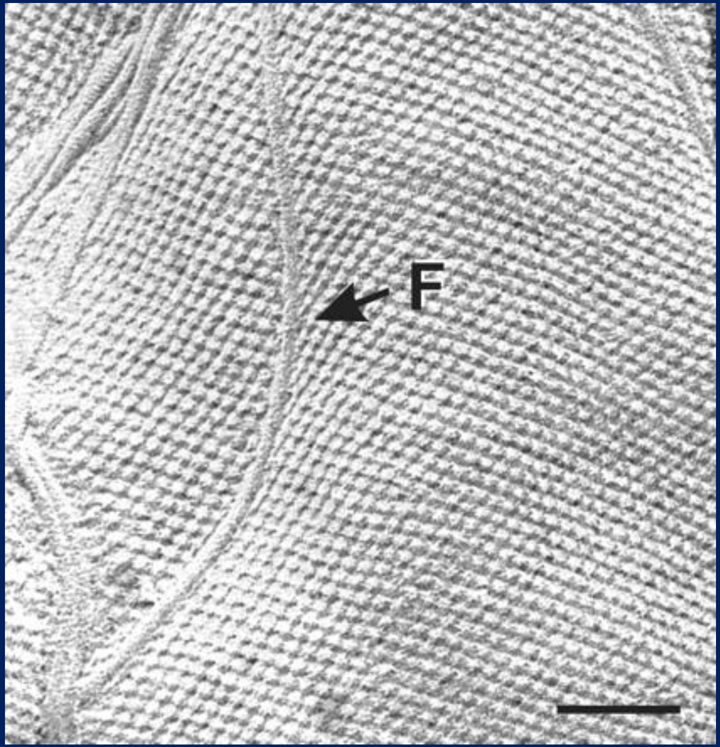
Synthesis of capsules



Capsule in Acinetobacter species by gram negative staining

Courtesy of Elliot Juni, Department of Microbiology and Immunology, The University of Michigan

протеиновые S-слои

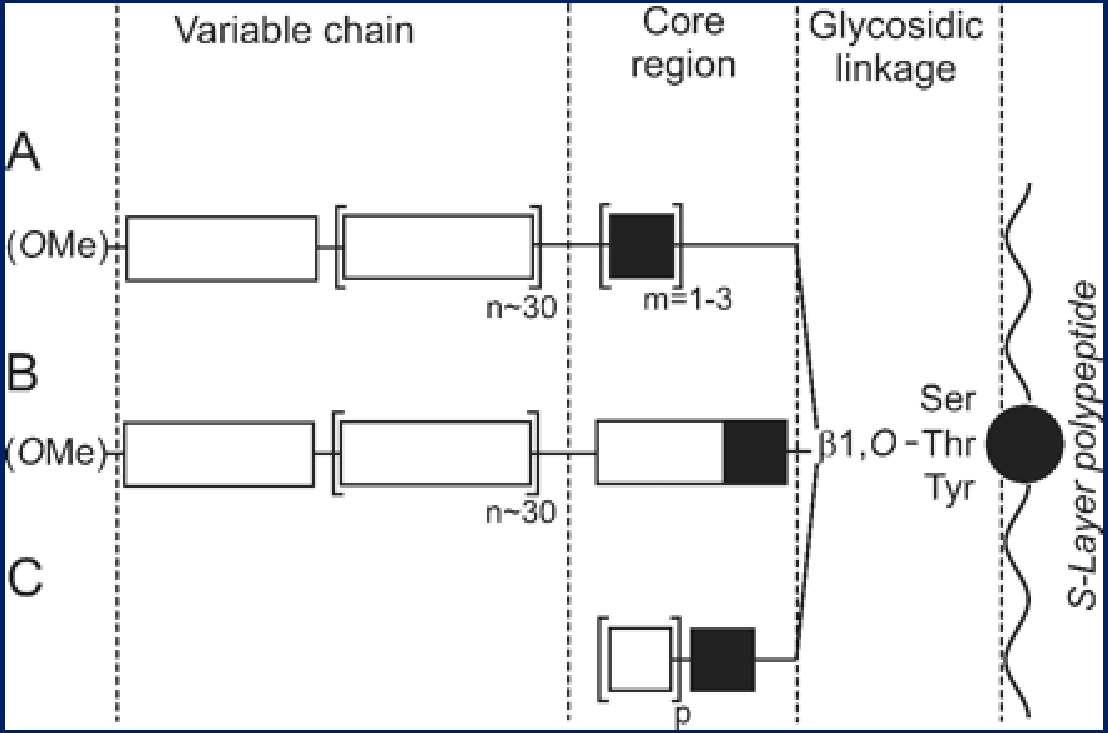


Гексагональная **белковая** решетка на поверхности клетки *Thermoanaerobacter thermohydrosulfuricus*

F – flagellum

bar - 100 nm

/Electron micrograph/

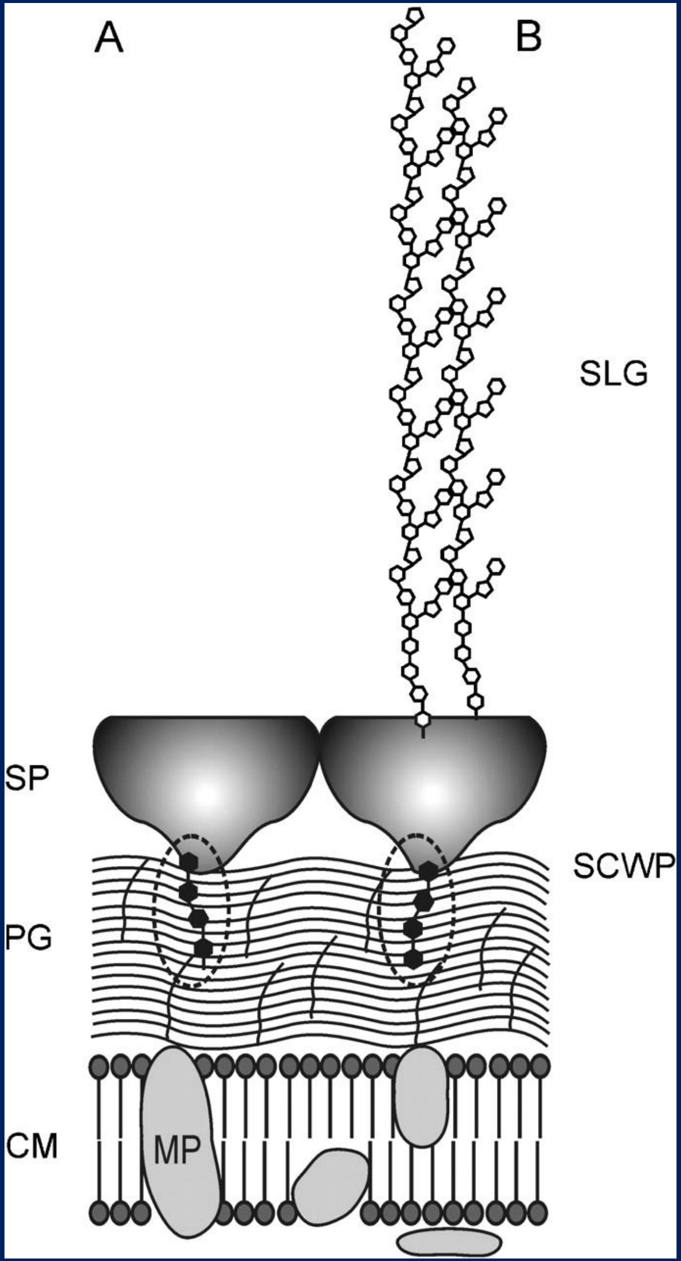


Строение **гликопротеинов** S-слоя в *Bacteria*

Заштрихованные квадраты = глюкоза (в семействе *Bacillaceae* обычно ß-)

Круг = гликозилированная АК в белке S-слоя

n – число повторов единиц
m – число коровых глюкоз
p – число одиночных глюкоз в укороченных гликанах

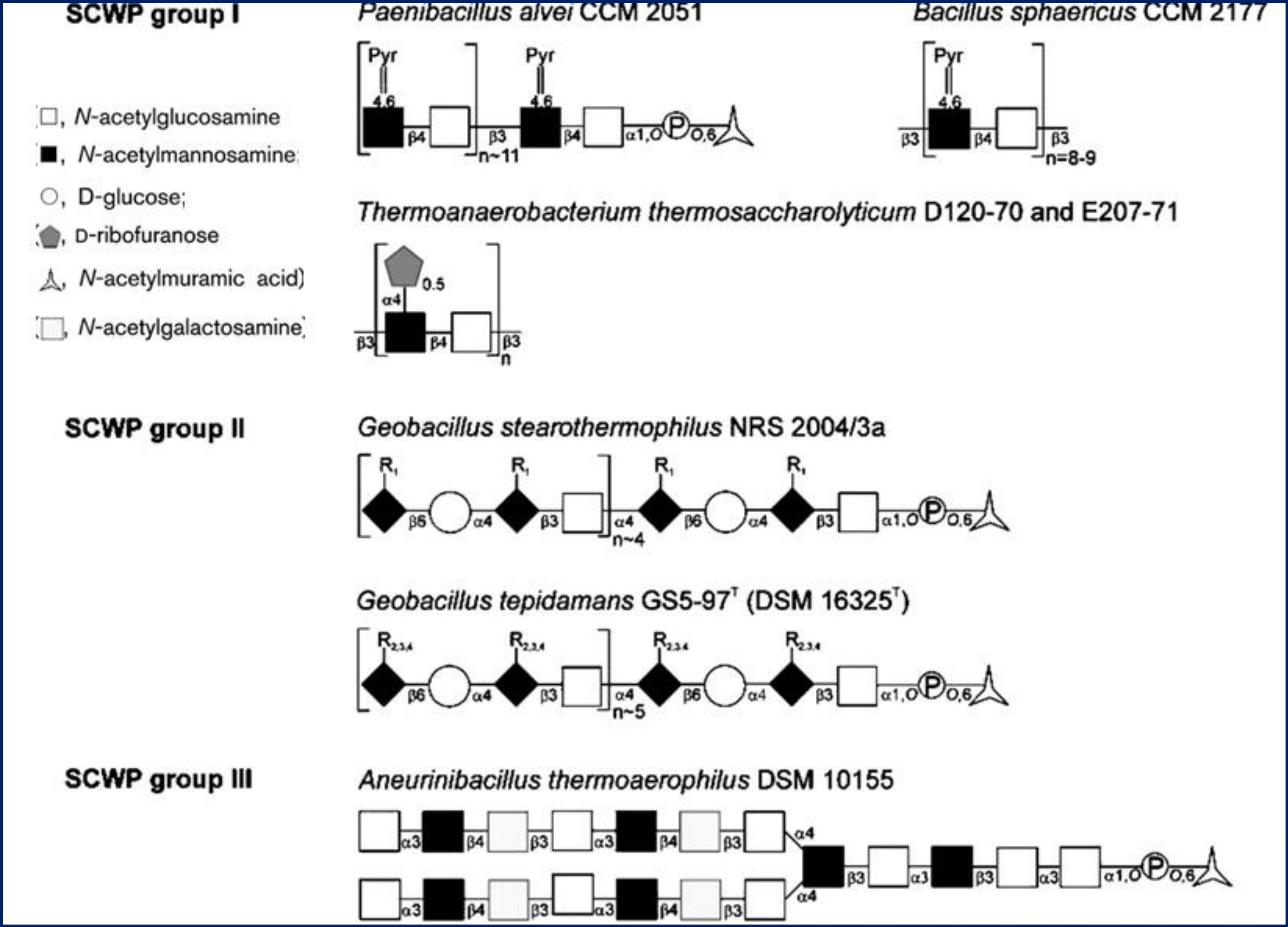


Профиль клеточной стенки с (А) белковым S-слоем и (В) гликопротеиновым S-слоем в Gram+ bacilli

Очерченные овалы – SCWP, ковалентно пришитые к мурамовой кислоте пептидогликана и нековалентно присоединенные к белку S-слоя.

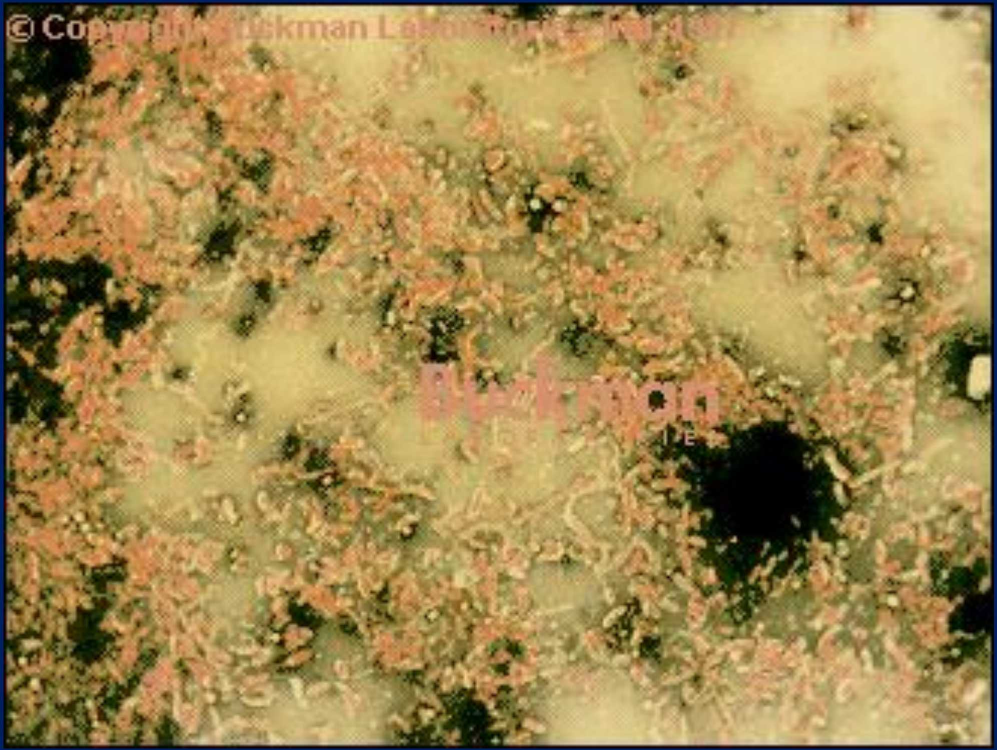
- CM – цитоплазм.мембрана
- MP – мембранный белок
- PG - peptidoglycan
- SP - S-layer protein
- SLG - S-layer glycoprotein glycan
- SCWP - secondary cell wall polymer

Structures of SCWPs of S-layer carrying *Bacillaceae.*

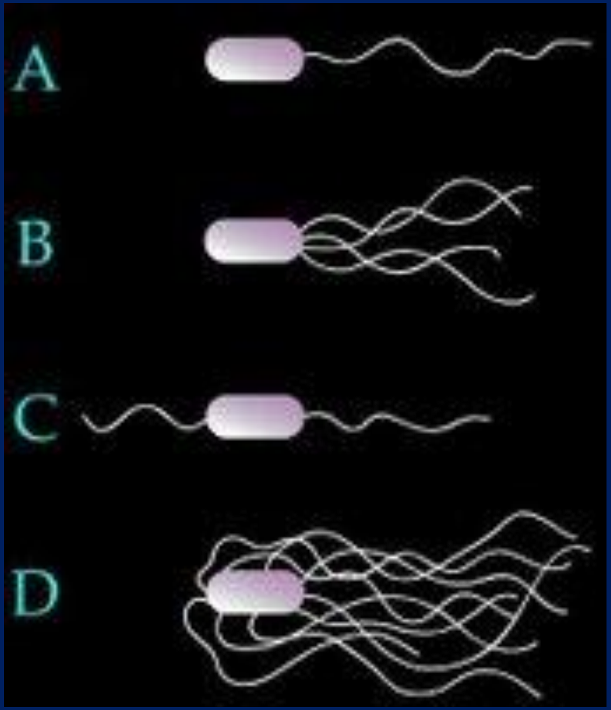

SCWP group I
□, N-acetylglucosamine
■, N-acetylmannosamine;
○, D-glucose;
⬟, D-ribofuranose
⅄, N-acetylmuramic acid)
□, N-acetylgalactosamine;
Paenibacillus alvei CCM 2051
Pyr
4,6
β4
β3
n~11
α1,O
P
O,6
Bacillus sphaericus CCM 2177
n=8-9
Thermoanaerobacterium thermosaccharolyticum D120-70 and E207-71
0.5
α4
n
SCWP group II
Geobacillus stearothermophilus NRS 2004/3a
R1
β6
n~4
Geobacillus tepidamans GS5-97T (DSM 16325T)
R2,3,4
n~5
SCWP group III
Aneurinibacillus thermoaerophilus DSM 10155
α3

Cell Surface Layers

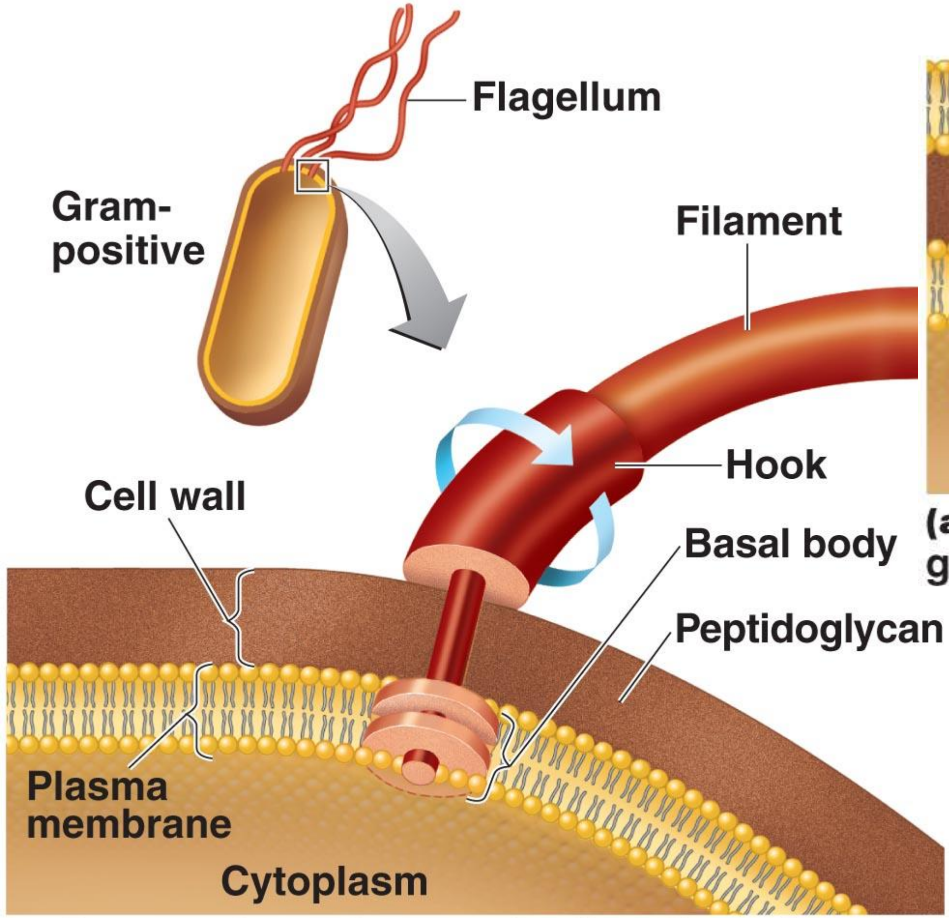
- slime layer
  - disorganized and loosely attached



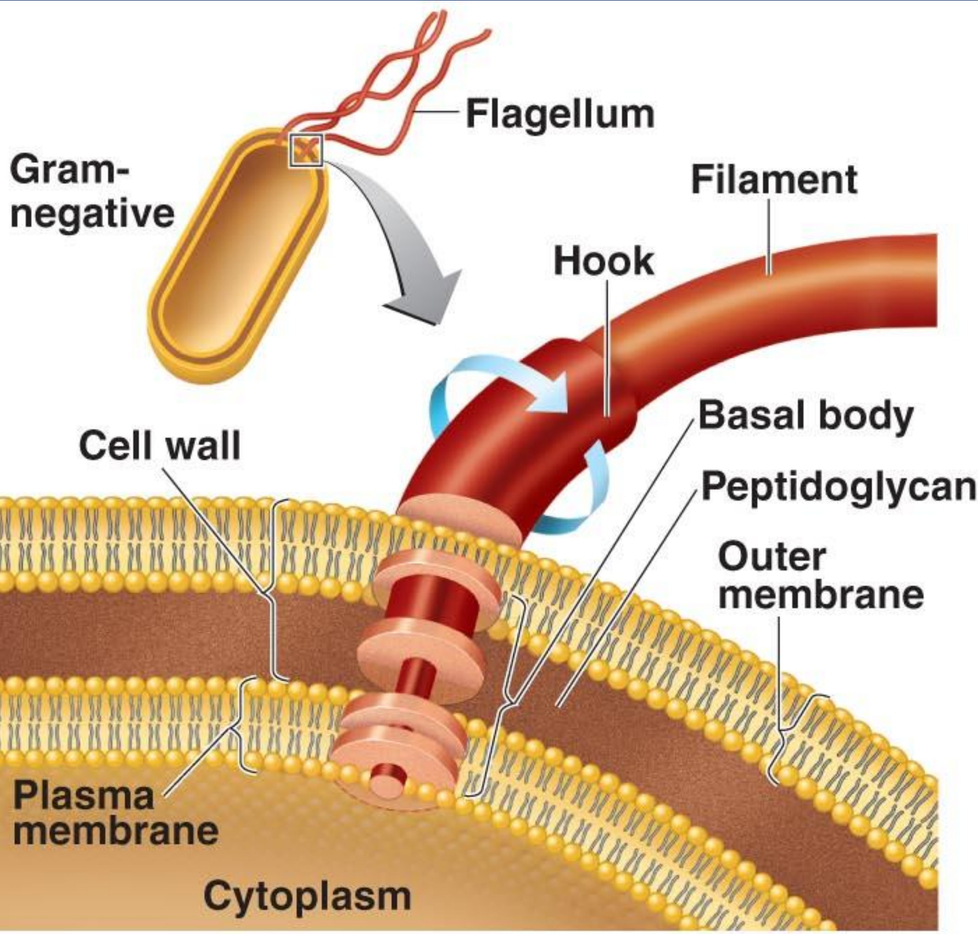
Синтез бактериального жгутика



gram+



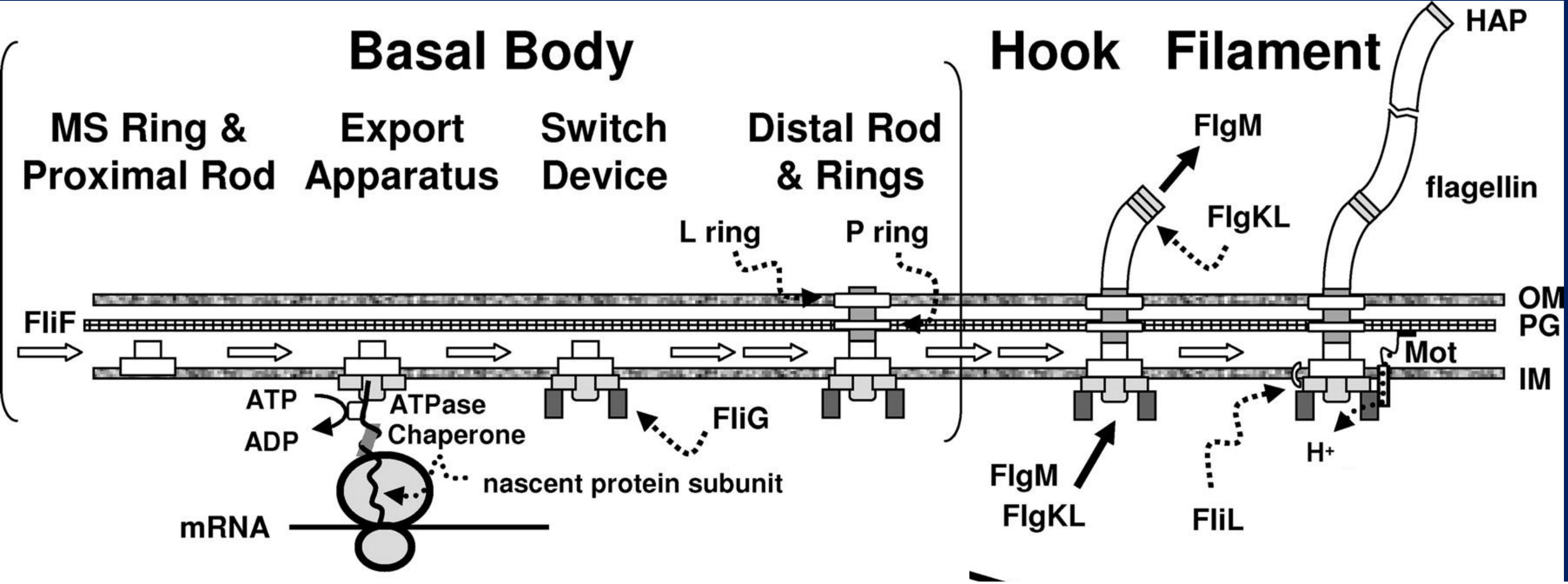
(b) Parts and attachment of a flagellum of a gram-positive bacterium

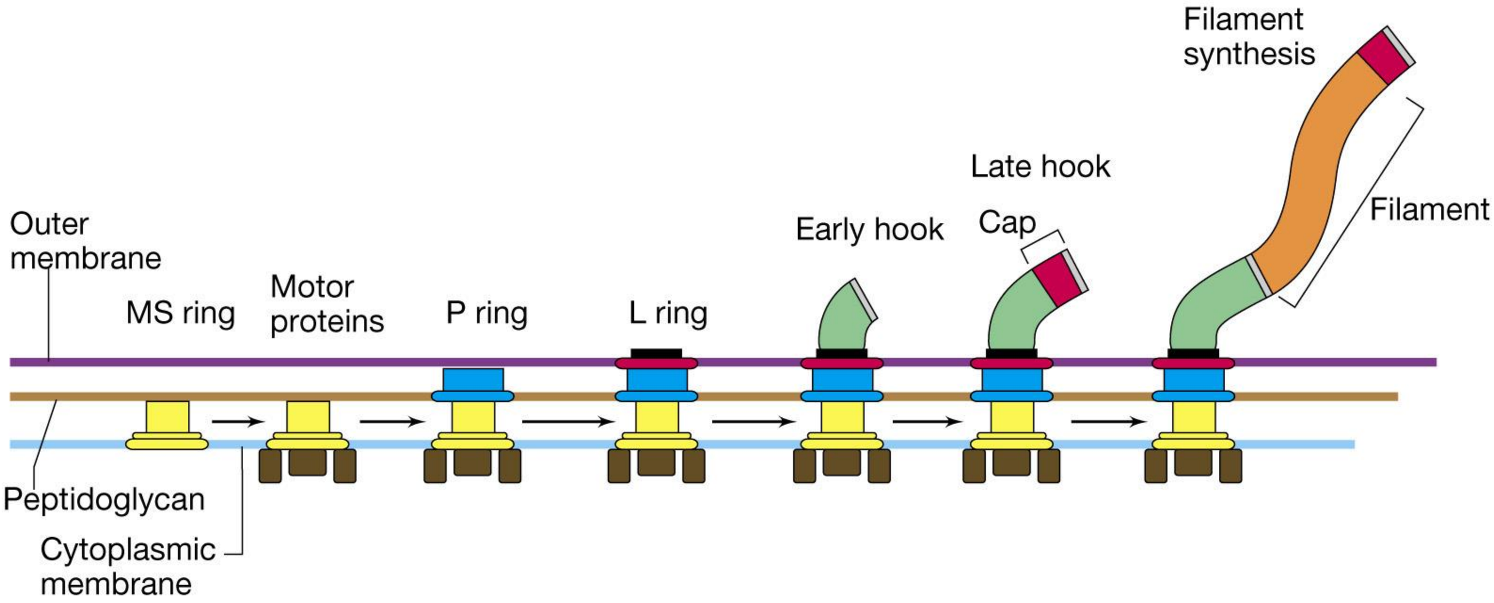


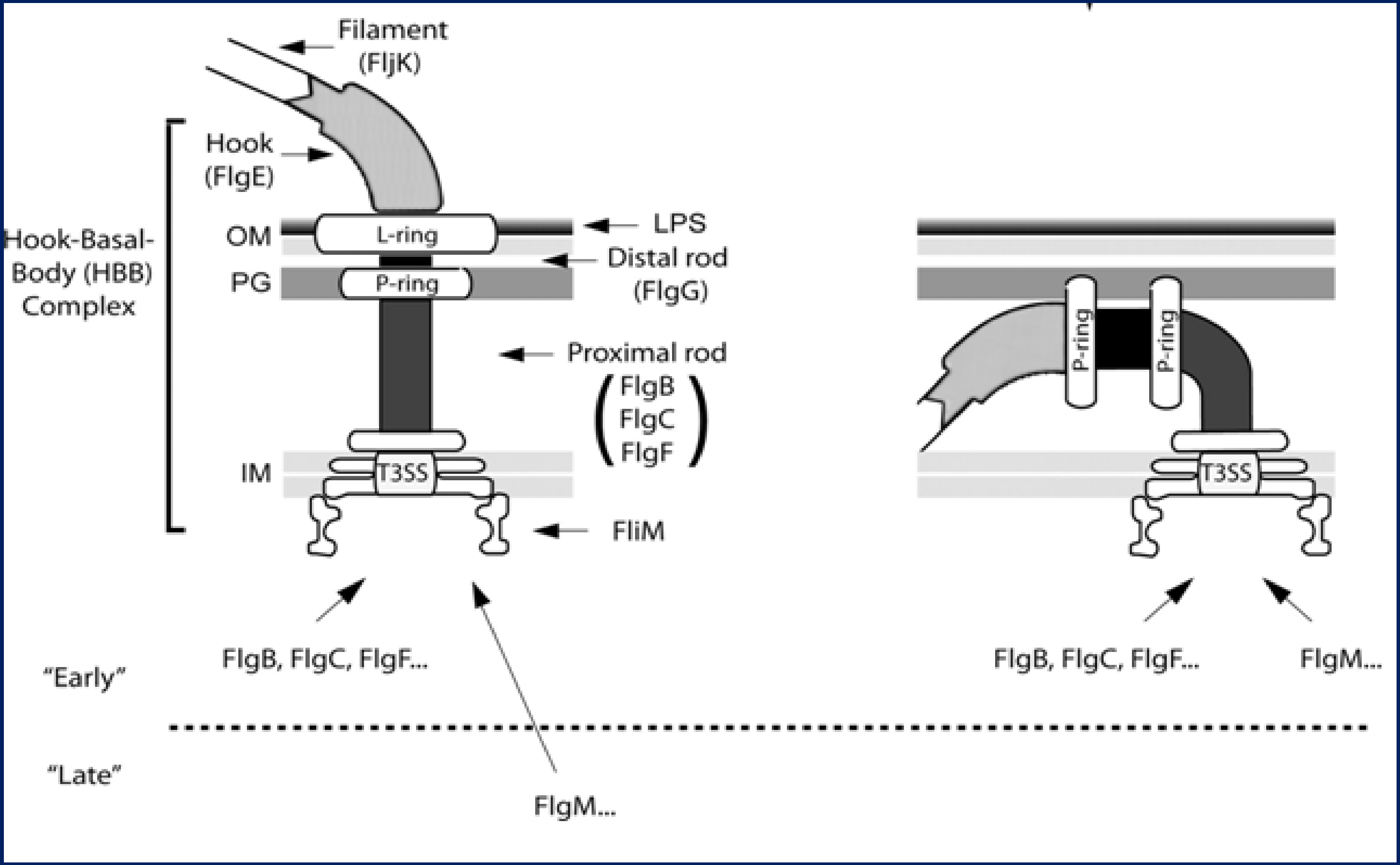
(a) Parts and attachment of a flagellum of a gram-negative bacterium

gram-

Синтез и сборка бактериального жгутика



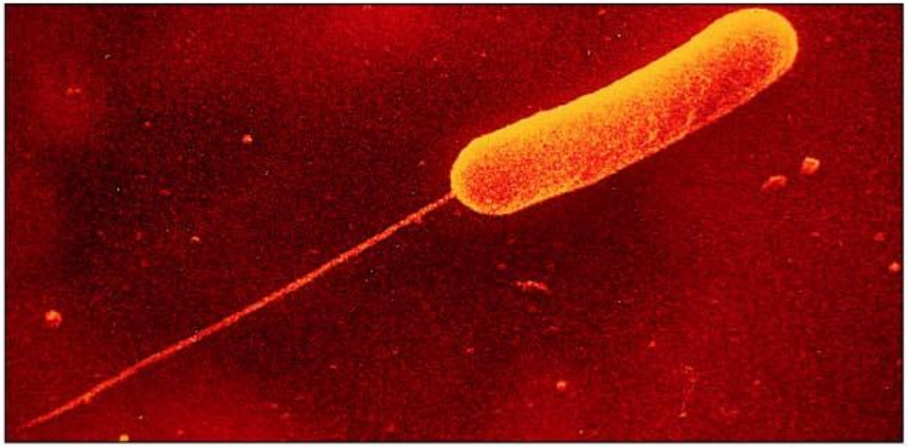




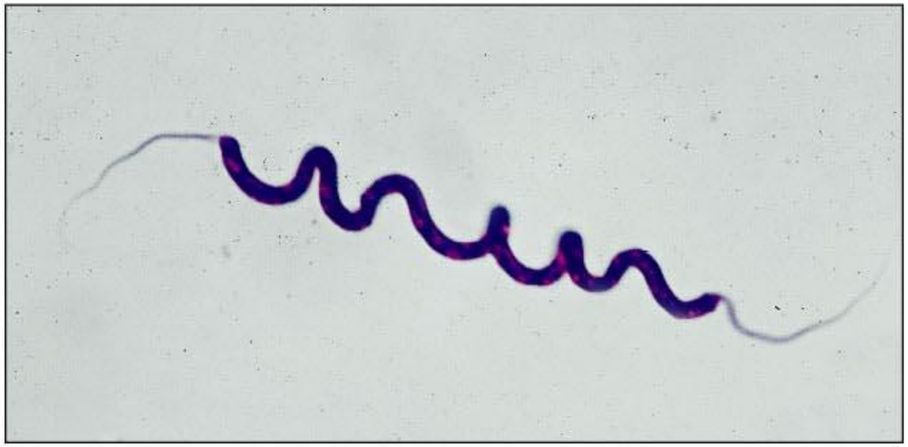
The completed flagellum traverses the inner membrane (IM), the peptidoglycan layer (PG), and the outer membrane (OM) comprising lipopoly-saccharide (LPS) in the outer leaflet.

Mutations in FlgG affect the conformational switch. Rod elongation affect the timing of substrate secretion. This results in formation of a periplasmic flagellum in FlgG point mutants

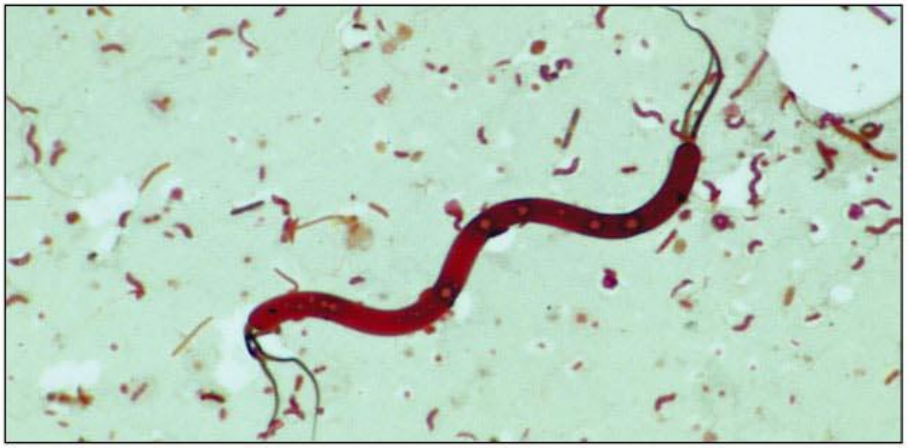
Arrangements of Bacterial Flagella



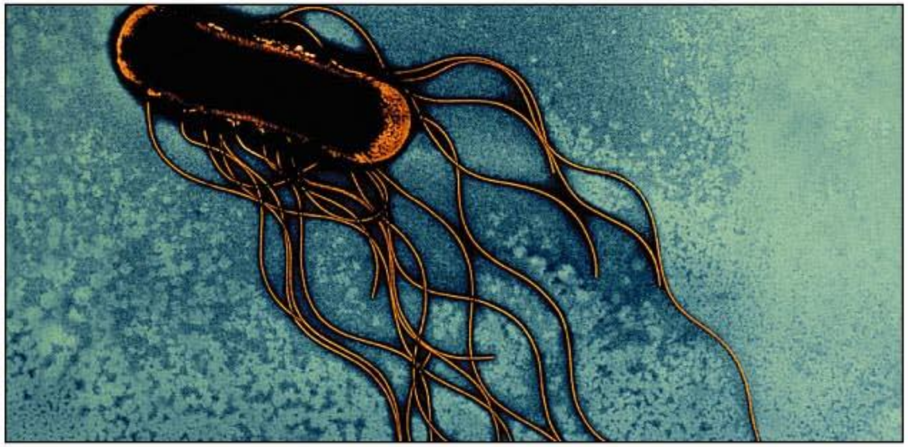
(a) Monotrichous SEM 1 µm



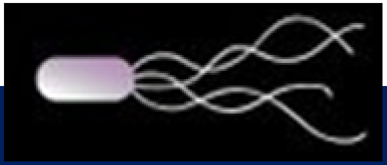
(b) Amphitrichous SEM 10 µm



(c) Lophotrichous SEM 1 µm



(d) Peritrichous SEM 1 µm



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